



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁶ : C12N 15/53, 9/02, 15/80, D21C 5/00, A61K 7/06, C12P 7/22, C12N 1/19, C09B 69/10 // (C12N 1/19, C12R 1:66)</p>	<p>A1</p>	<p>(11) International Publication Number: WO 95/07988 (43) International Publication Date: 23 March 1995 (23.03.95)</p>
<p>(21) International Application Number: PCT/US94/10264 (22) International Filing Date: 13 September 1994 (13.09.94) (30) Priority Data: 08/122,230 17 September 1993 (17.09.93) US 08/122,827 17 September 1993 (17.09.93) US 08/162,827 3 December 1993 (03.12.93) US 08/172,331 22 December 1993 (22.12.93) US (71) Applicants: NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK). NOVO NORDISK BIOTECH, INC. [US/US]; 1445 Drew Avenue, Davis, CA 95616-4880 (US). (72) Inventors: WAHLEITHNER, Jill, Angela; 1718 Tea Place, Davis, CA 95616 (US). CHRISTENSEN, Bjørn, Eggert; Dronninggaards Allé 32, DK-2840 Holte (DK). SCHNEIDER, Palle; Rydtoften 43, DK-2750 Ballerup (DK). (74) Agents: ZELSON, Steve, T. et al.; Novo Nordisk of North America, Inc., Suite 6400, 405 Lexington Avenue, New York, NY 10174 (US).</p>		<p>(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, NO, NZ, PL, RO, RU, SD, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>
<p>(54) Title: PURIFIED pH NEUTRAL RHIZOCTONIA LACCASES AND NUCLEIC ACIDS ENCODING SAME (57) Abstract The present invention relates to isolated nucleic acid fragments containing a sequence encoding a <i>Rhizoctonia solani</i> laccase having optimum activity at a neutral or basic pH, and the laccase proteins encoded thereby.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

PURIFIED PH NEUTRAL RHIZOCTONIA LACCASES AND NUCLEIC
ACIDS ENCODING SAME

5

Related Applications

This application is a continuation-in-part of co-
pending U.S. Serial Nos. 08/122,230, 08/122,827, and
08/162,827, the contents of which are incorporated by
10 reference in their entirety.

Field of the Invention

The present invention relates to isolated nucleic acid
fragments encoding a fungal oxidoreductase enzyme and the
15 purified enzymes produced thereby. More particularly, the
invention relates to nucleic acid fragments encoding a
phenol oxidase, specifically a laccase, which functions at
a neutral pH.

20 Background of the Invention

Laccases (benzenediol:oxygen oxidoreductases) are
multi-copper containing enzymes that catalyze the oxidation
of phenolics. Laccase-mediated oxidations result in the
production of aryloxy-radical intermediates from suitable
25 phenolic substrate; the ultimate coupling of the
intermediates so produced provides a combination of dimeric,
oligomeric, and polymeric reaction products. Such reactions
are important in nature in biosynthetic pathways which lead
to the formation of melanin, alkaloids, toxins, lignins, and
30 humic acids. Laccases are produced by a wide variety of
fungi, including ascomycetes such as *Aspergillus*,
Neurospora, and *Podospora*, the deuteromycete *Botrytis*, and

basidiomycetes such as *Collybia*, *Fomes*, *Lentinus*, *Pleurotus*,
Trametes, and perfect forms of *Rhizoctonia*. Laccase
exhibits a wide range of substrate specificity, and each
different fungal laccase usually differs only quantitatively
5 from others in its ability to oxidize phenolic substrates.
Because of the substrate diversity, laccases generally have
found many potential industrial applications. Among these
are lignin modification, paper strengthening, dye transfer
inhibition in detergents, phenol polymerization, juice
10 manufacture, phenol resin production, and waste water
treatment.

Although the catalytic capabilities are similar,
laccases made by different fungal species do have different
temperature and pH optima, and these may also differ
15 depending on the specific substrate. A number of these
fungal laccases have been isolated, and the genes for
several of these have been cloned. For example, Choi et
al. (*Mol. Plant-Microbe Interactions* 5: 119-128, 1992)
describe the molecular characterization and cloning of the
20 gene encoding the laccase of the chestnut blight fungus,
Cryphonectria parasitica. Kojima et al. (*J. Biol. Chem.*
265: 15224-15230, 1990; JP 2-238885) provide a description
of two allelic forms of the laccase of the white-rot
basidiomycete *Coriolus hirsutus*. Germann and Lerch
25 (*Experientia* 41: 801, 1985; *PNAS USA* 83: 8854-8858, 1986)
have reported the cloning and partial sequencing of the
Neurospora crassa laccase gene. Saloheimo et al. (*J. Gen.*
Microbiol. 137: 1537-1544, 1985; WO 92/01046) have
disclosed a structural analysis of the laccase gene from the
30 fungus *Phlebia radiata*. However, virtually all of the
known fungal laccases function best at acidic pHs (e.g.,
between pH 3.0 and 6.0), and are typically inactive at

neutral or basic pHs. Since a number of the aforesaid potential industrial methods are preferentially conducted at neutral or basic pH, most fungal laccases perform poorly in such methods. Thus, the available fungal laccases are
5 inadequate for application in a number of important commercial methods.

An exception to this rule is the extracellular laccase produced by certain species of *Rhizoctonia*. Bollag et al. have reported a laccase with a pH optimum of about 7.0
10 produced by *Rhizoctonia praticola*. A laccase of this type would be far more useful in industrial methods requiring neutral pH than previously known laccases. However, the *R. praticola* enzyme was neither purified nor further characterized, nor, to date, has any other laccase having
15 this trait been purified or characterized. Moreover, although other laccase genes have been isolated, as described above, these have been genes encoding enzymes which function best at acidic pH. Recombinant production and commercially adequate yields of a pH neutral or basic
20 laccase have thus been unattainable due to the fact that neither the enzyme per se nor the laccase gene encoding such an enzyme has previously been isolated and/or purified and sequenced. The present invention now provides a solution to each of these problems.

25

Summary of the Invention

The present invention relates to an isolated nucleic acid fragment comprising a nucleic acid sequence encoding a *Rhizoctonia* laccase which functions optimally at a pH
30 between 6.0 to 8.5. By "functioning optimally" is meant that the enzyme exhibits significant (i.e., at least about 30% of maximum, preferably at least about 50%, and most

preferably from 50% to maximum) activity within the pH range of between about 6.0-8.5, as determined by activity in one or more standard laccase assays for substrates such as the syringaldazine, ABTS, 2,6-dimethoxyphenol, or 4
5 antiaminopyrine + N-ethyl-N-sulfobutyl-m-toluidine. A preferred substrate for the laccases of the present invention is syringaldazine. In a preferred embodiment, the laccase is a *Rhizoctonia solani* laccase. The invention also relates to a substantially pure laccase encoded by the novel
10 nucleic acid sequence. By "substantially pure" is meant a laccase which is essentially (i.e., ≥90%) free of other non-laccase proteins.

In order to facilitate production of the novel laccase, the invention also provides vectors and host cells
15 comprising the claimed nucleic acid fragment, which vectors and host cells are useful in recombinant production of the laccase. The nucleic acid fragment is operably linked to transcription and translation signals capable of directing expression of the laccase protein in the host cell of
20 choice. A preferred host cell is a fungal cell, most preferably of the genus *Aspergillus*. Recombinant production of the laccase of the invention is achieved by culturing a host cell transformed or transfected with the nucleic acid fragment of the invention, or progeny thereof, under
25 conditions suitable for expression of the laccase protein, and recovering the laccase protein from the culture.

The laccases of the present invention are useful in a number of industrial processes in which oxidation of phenolics is required. These processes include lignin
30 manipulation, juice manufacture, phenol polymerization and phenol resin production. In a preferred embodiment, the

enzyme of the invention is used in a process requiring a neutral or somewhat basic pH for greatest efficiency.

Brief Description of the Figures

5 Figure 1 illustrates the nucleotide and amino acid sequence of RSlac1. Lower case letters in the nucleotide sequence indicate the position of introns.

 Figure 2 illustrates the nucleotide and amino acid sequence of RSlac2. Lower case letters in the nucleotide
10 sequence indicate the position of introns.

 Figure 3 illustrates a restriction map of the plasmid pMWR-1.

 Figure 4 illustrates the nucleotide and amino acid sequence of the translated region of RSlac3.

15 Figure 5 illustrates the syringaldazine oxidase activity of RSlac1 (90mM buffer, 20 μ M syringaldazine, 20°C).

 Figure 6 illustrates the syringaldazine oxidase activity of RSlac2 (93mM buffer, 20 μ M syringaldazine,
20 20°C).

Detailed Description of the Invention

 Certain species of the genus *Rhizoctonia* have been reported as producing laccase; therefore, an initial search focused on identifying the presence of these enzymes in
25 various *Rhizoctonia solani* isolates. Samples are cultured and the supernatants periodically analyzed for the presence of laccase by the ABTS method, described below. Laccase is observed in all the *Rhizoctonia* cultures. Harvested laccases are electrophoretically separated and stained with
30 ABTS. One isolate, RS22, produces a laccase with a basic pI, and is selected for further study.

The remaining studies focus on purification and characterization of the enzyme from RS22. Briefly, the fermentation broth is filtered and concentrated by UF with a membrane cut off of about 10,000. A first ion exchange chromatography step is conducted at pH 4.5 in acetate buffer, with step elution using NaCl. The eluate is then ultrafiltered and rechromatographed, and eluted with a NaCl gradient. Active fractions are pooled for further study.

The intact protein thus isolated and purified (hereinafter referred to as RSlac3) is first subjected to partial sequencing, and the N-terminal sequence obtained is as follows:

AVRNYKFDIKNVNVAPDGFQRPIVSV (SEQ. ID. NO.: 5)

The protein is further subjected to digestion with a lysine- or glutamic-acid specific protease, and additional peptides obtained from the protein have the following sequences, which can be aligned with sequences in *Coriolus hirsutus*:

Peptide 1:

20 SQYVDGLRGPLVIYDPDDDH (SEQ. ID. NO: 6)

Peptide 2:

GLALVFAEAPSQIRQGVQSVQPDDA (SEQ. ID. NO.: 7)

Peptide 3:

SRVBVBASTVVMLEBWYHTPAXVLE (SEQ. ID. NO. 8)

25 Peptide 4:

SLGPTPNYVNPXIRDVVRVGTTVV (SEQ. ID. NO. 9)

The following peptides are also found, but do not correspond to *Coriolus* sequences

Peptide 5:

30 IRYVGGPVAVX(N?)RSVI (SEQ. ID. NO.: 10)

Peptide 6:

ILANPA (SEQ. ID. NO.: 11)

Peptide 7:

YEAPSLPT (SEQ. ID. NO.: 12)

In the above sequences, B designates a residue which is either aspartic acid or asparagine, and X designates
5 unidentified residues.

In order to initiate screening for a *Rhizoctonia* laccase gene, an *R. solani* genomic library is prepared. Total DNA is partially digested with restriction enzyme Sau3A, and electrophoresed in an agarose gel to isolate DNA
10 fragments between 8 and 21 kb in size. The fractionated fragments are ligated to λ phage EMBL3 arms with BamHI ends, and the resulting phage packaged in vitro. These phage are used as a library to create a library of 170,000 plaques in *E. coli* and amplified 100-fold for future use.

15 In order to develop probes for isolation of the *R. solani* laccase gene, the protein sequences of five known laccases are analyzed to determine consensus sequences, and two degenerate oligonucleotides constructed based on observed consensus sequences (Choi et al. *supra*; Germann and
20 Lerch, *supra*; Saloheimo et al, *supra*, Kojima et al, *supra*). These oligos are mixed with *R. solani* genomic DNA and a DNA fragment of 220 nucleotide fragment is amplified using a taq polymerase chain reaction(PCR). The 220-nucleotide fragment is then cloned into plasmid vector.

25 The PCR fragment is used as a probe to screen 25,000 plaques from the amplified genomic library. Positive clones from this screen fall into two classes that are subsequently shown, by DNA sequence analysis, to code for two different laccase genes, *RSlac1* and *RSlac2*. The nucleotide sequence
30 for each of these genes (SEQ ID. NOS.: 1 and 3), and the predicted amino acid sequence for each protein (SEQ. ID. NOS.: 2 and 4), are presented in, respectively, Figures 1

and 2. The homology between the two sequences is approximately 63%. Compared to known laccase sequences from *Coriolus hirsutus*, *Phlebia radiata*, *Aspergillus nidulans*, *Cryphonectria parasitica*, and *Neurospora crassa*, the RS laccases show between about 30-40% homology. Each of the two coding sequences is cloned into an expression vector operably linked to *Aspergillus oryzae* taka-amylase transcription and translation signals (See Figure 3). Each of the two laccase expression vectors is transformed into an *Aspergillus oryzae* and *Aspergillus niger* host cell, and the host cells screened for the presence of laccase.

For isolation of the *RSlac3* gene, polyA RNA is purified from *R. solani* mycelia grown in the presence of anisidine. The RNA is used as a template for cDNA synthesis. The cDNA is fractionated and fragments between 1.7-3.5 kb collected, and a cDNA library created by cloning the fractionated DNA into a yeast vector. 3000 transformants from this library are screened on ABTS. After 24 hours, a single colony appears positive. The plasmid from the colony is isolated and the insert sequenced. Portions of the predicted amino acid sequence correspond with the sequences of the fragments obtained from RS 22, described *supra*. The complete nucleotide and amino acid sequences are depicted in Figure 4, and in SEQ. ID. NOS.: 13 and 14, respectively. *RSlac3* shows 48% homology with *RSlac1* and 50% homology with *RSlac2*. *RSlac3* also shows 48% homology with the *Coriolus hirsutus* laccase gene.

According to the invention, a *Rhizoctonia* gene encoding a pH neutral or basic laccase can be obtained by methods described above, or any alternative methods known in the art, using the information provided herein. The gene can be expressed, in active form, using an expression

vector. A useful expression vector contains an element that permits stable integration of the vector into the host cell genome or autonomous replication of the vector in a host cell independent of the genome of the host cell, and preferably one or more phenotypic markers which permit easy selection of transformed host cells. The expression vector may also include control sequences encoding a promoter, ribosome binding site, translation initiation signal, and, optionally, a repressor gene or various activator genes. To permit the secretion of the expressed protein, nucleotides encoding a signal sequence may be inserted prior to the coding sequence of the gene. For expression under the direction of control sequences, a laccase gene to be treated according to the invention is operably linked to the control sequences in the proper reading frame. Promoter sequences that can be incorporated into plasmid vectors, and which can direct the transcription of the laccase gene, include but are not limited to the prokaryotic β -lactamase promoter (Villa-Kamaroff, et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731) and the tac promoter (DeBoer, et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25). Further references can also be found in "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; and in Sambrook et al., Molecular Cloning, 1989.

25

The expression vector carrying the DNA construct of the invention may be any vector which may conveniently be subjected to recombinant DNA procedures, and the choice of vector will typically depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, i.e. a vector which exists as an extrachromosomal entity, the replication of which is

30

independent of chromosomal replication, e.g. a plasmid, or an extrachromosomal element, minichromosome or an artificial chromosome. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host
5 cell genome and replicated together with the chromosome(s) into which it has been integrated.

In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may
10 be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA construct of the invention,
15 especially in a bacterial host, are the promoter of the *lac* operon of *E.coli*, the *Streptomyces coelicolor* agarase gene *dagA* promoters, the promoters of the *Bacillus licheniformis* α -amylase gene (*amyL*), the promoters of the *Bacillus stearothermophilus* maltogenic amylase gene (*amyM*), the
20 promoters of the *Bacillus amyloliquefaciens* α -amylase (*amyQ*), or the promoters of the *Bacillus subtilis* *xylA* and *xylB* genes. In a yeast host, a useful promoter is the *eno-1* promoter. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding *A.*
25 *oryzae* TAKA amylase, *Rhizomucor miehei* aspartic proteinase, *A. niger* neutral α -amylase, *A. niger* acid stable α -amylase, *A. niger* or *A. awamsii* glucoamylase (*gluA*), *Rhizomucor miehei* lipase, *A. oryzae* alkaline protease, *A. oryzae* triose phosphate isomerase or *A. nidulans* acetamidase. Preferred
30 are the TAKA-amylase and *gluA* promoters.

The expression vector of the invention may also comprise a suitable transcription terminator and, in eukaryotes, polyadenylation sequences operably connected to the DNA sequence encoding the laccase of the invention.

5 Termination and polyadenylation sequences may suitably be derived from the same sources as the promoter. The vector may further comprise a DNA sequence enabling the vector to replicate in the host cell in question. Examples of such sequences are the origins of replication of plasmids pUC19,
10 pACYC177, pUB110, pE194, pAMB1 and pIJ702.

The vector may also comprise a selectable marker, e.g. a gene the product of which complements a defect in the host cell, such as the *dal* genes from *B. subtilis* or *B. li-*
15 *cheniformis*, or one which confers antibiotic resistance such as ampicillin, kanamycin, chloramphenicol or tetracycline resistance. Examples of *Aspergillus* selection markers include *amdS*, *pyrG*, *argB*, *niaD* and *sc*, a marker giving rise to hygromycin resistance. Preferred for use in an
20 *Aspergillus* host cell are the *amdS* and *pyrG* markers of *A. nidulans* or *A. oryzae*. A frequently used mammalian marker is the dihydrofolate reductase (DHFR) gene. Furthermore, selection may be accomplished by co-transformation, e.g. as described in WO 91/17243.

25

It is generally preferred that the expression is extracellular. The laccases of the present invention may thus comprise a preregion permitting secretion of the expressed protein into the culture medium. If desirable,
30 this preregion may be native to the laccase of the invention or substituted with a different preregion or signal sequence, conveniently accomplished by substitution of the

DNA sequences encoding the respective preregions. For example, the preregion may be derived from a glucoamylase or an amylase gene from an *Aspergillus* species, an amylase gene from a *Bacillus* species, a lipase or proteinase gene from

5 *Rhizomucor miehei*, the gene for the α -factor from *Saccharomyces cerevisiae* or the calf prochymosin gene. Particularly preferred, when the host is a fungal cell, is the preregion for *A. oryzae* TAKA amylase, *A. niger* neutral amylase, the maltogenic amylase form *Bacillus* NCIB 11837, *B.*

10 *stearothermophilus* α -amylase, or *Bacillus licheniformis* subtilisin. An effective signal sequence is the *A. oryzae* TAKA amylase signal, the *Rhizomucor miehei* aspartic proteinase signal and the *Rhizomucor miehei* lipase signal.

15 The procedures used to ligate the DNA construct of the invention, the promoter, terminator and other elements, respectively, and to insert them into suitable vectors containing the information necessary for replication, are well known to persons skilled in the art (cf., for instance,

20 Sambrook et al. *Molecular Cloning*, 1989).

The cell of the invention either comprising a DNA construct or an expression vector of the invention as defined above is advantageously used as a host cell in the

25 recombinant production of a enzyme of the invention. The cell may be transformed with the DNA construct of the invention, conveniently by integrating the DNA construct in the host chromosome. This integration is generally considered to be an advantage as the DNA sequence is more

30 likely to be stably maintained in the cell. Integration of the DNA constructs into the host chromosome may be performed

according to conventional methods, e.g. by homologous or heterologous recombination. Alternatively, the cell may be transformed with an expression vector as described above in connection with the different types of host cells.

5

The host cell may be selected from prokaryotic cells, such as bacterial cells. Examples of suitable bacteria are gram positive bacteria such as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus lentus*, *Bacillus brevis*, *Bacillus*
10 *stearothermophilus*, *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus coagulans*, *Bacillus circulans*, *Bacillus lautus*, *Bacillus megaterium*, *Bacillus thuringiensis*, or *Streptomyces lividans* or *Streptomyces murinus*, or gram negative bacteria such as *E.coli*. The
15 transformation of the bacteria may for instance be effected by protoplast transformation or by using competent cells in a manner known per se.

The host cell may also be a eukaryote, such as mammalian cells, insect cells, plant cells or preferably
20 fungal cells, including yeast and filamentous fungi. For example, useful mammalian cells include CHO or COS cells. A yeast host cell may be selected from a species of *Saccharomyces* or *Schizosaccharomyces*, e.g. *Saccharomyces cerevisiae*. Useful filamentous fungi may be selected from a
25 species of *Aspergillus*, e.g. *Aspergillus oryzae* or *Aspergillus niger*. Alternatively, a strain of a *Fusarium* species, e.g. *F. oxysporum*, can be used as a host cell. Fungal cells may be transformed by a process involving protoplast formation and transformation of the protoplasts followed by regeneration of the cell wall in a manner known per
30 se. A suitable procedure for transformation of *Aspergillus* host cells is described in EP 238 023. A suitable method of

transforming *Fusarium* species is described by Malardier et al., 1989.

The present invention thus provides a method of producing a recombinant laccase of the invention, which method comprises cultivating a host cell as described above under conditions conducive to the production of the enzyme and recovering the enzyme from the cells and/or culture medium. The medium used to cultivate the cells may be any conventional medium suitable for growing the host cell in question and obtaining expression of the laccase of the invention. Suitable media are available from commercial suppliers or may be prepared according to published formulae (e.g. in catalogues of the American Type Culture Collection).

The resulting enzyme may be recovered from the medium by conventional procedures including separating the cells from the medium by centrifugation or filtration, precipitating the proteinaceous components of the supernatant or filtrate by means of a salt, e.g. ammonium sulphate, followed by purification by a variety of chromatographic procedures, e.g. ion exchange chromatography, gel filtration chromatography, affinity chromatography, or the like. Preferably, the isolated protein is about 90% pure as determined by SDS-PAGE, purity being most important in food, juice or detergent applications.

In a particularly preferred embodiment, the expression of laccase is achieved in a fungal host cell, such as *Aspergillus*. As described in detail in the following examples, the laccase gene is ligated into a plasmid containing the *Aspergillus oryzae* TAKA α -amylase promoter, and the *Aspergillus nidulans* *amdS* selectable marker. Alternatively, the *amdS* may be on a separate plasmid and

used in co-transformation. The plasmid (or plasmids) is used to transform an *Aspergillus* species host cell, such as *A. oryzae* or *A. niger* in accordance with methods described in Yelton et al. (PNAS USA 81: 1470-1474, 1984).

5 Those skilled in the art will recognize that the invention is not limited to use of the nucleic acid fragments specifically disclosed herein, for example, in Figures 1 and 2. It will be apparent that the invention also encompasses those nucleotide sequences that encode the
10 same amino acid sequences as depicted in Figures 1, 2 and 3, but which differ from those specifically depicted nucleotide sequences by virtue of the degeneracy of the genetic code. In addition, the invention also encompasses other nucleotide fragments, and the proteins encoded thereby, which encode
15 laccase proteins having substantially the same pH optimum as those of *Rhizoctonia solani*, and which show a significant level of homology with the *Rhizoctonia solani* amino acid sequence. For example, the present data show that more than one species of *Rhizoctonia* produces a laccase with the
20 desired pH profile; it is therefore expected that other *Rhizoctonia* species also produce similar laccases and therefore, using the technology described herein, can be used as a source for genes within the scope of the claimed invention. As also shown in the present examples, not only
25 is there more than one nucleotide and amino acid sequence that encodes a laccase with the required characteristics, there is also considerable variation tolerated within the sequence while still producing a functional enzyme. Therefore, the invention also encompasses any variant
30 nucleotide sequence, and the protein encoded thereby, which protein retains at least about an 80% homology with one or the other of the amino acid sequences depicted in Figures 1,

2 and 3, and retains both the laccase and pH optimum activity of the sequences described herein. In particular, variants which retain a high level (i.e., $\geq 80\%$) of homology at highly conserved regions of the *Rhizoctonia* laccase are contemplated. Such regions are identified as residues 458-469 in RSLAC1, and 478-489 in RSLAC2; and residues 131-144 in RSLAC1 and 132-145 in RSLAC2.

Useful variants within the categories defined above include, for example, ones in which conservative amino acid substitutions have been made, which substitutions do not significantly affect the activity of the protein. By conservative substitution is meant that amino acids of the same class may be substituted by any other of that class. For example, the nonpolar aliphatic residues Ala, Val, Leu, and Ile may be interchanged, as may be the basic residues Lys and Arg, or the acidic residues Asp and Glu. Similarly, Ser and Thr are conservative substitutions for each other, as are Asn and Gln. It will be apparent to the skilled artisan that such substitutions can be made outside the regions critical to the function of the molecule and still result in an active enzyme. Retention of the desired activity can readily be determined by conducting a standard ABTS oxidation method in 0.1M sodium phosphate at pH 7.0.

The protein can be used in number of different industrial processes; although the enzyme is also functional to some extent at lower pH, the *R. solani* laccase is most beneficially used in processes that are usually conducted at a neutral or alkaline pH, since other laccases are not active in this pH range. These processes include polymerization of lignin, both Kraft and lignosulfates, in solution, in order to produce a lignin with a higher molecular weight. A neutral/alkaline laccase is a

particular advantage in that Kraft lignin is more soluble at higher pHs. Such methods are described in, for example, Jin et al., *Holzforschung* 45(6): 467-468, 1991; US Patent No. 4,432,921; EP 0 275 544; PCT/DK93/00217, 1992.

5 The laccase of the present invention can also be used for in-situ depolymerization of lignin in Kraft pulp, thereby producing a pulp with lower lignin content. This use of laccase is an improvement over the current use of chlorine for depolymerization of lignin, which leads to the
10 production of chlorinated aromatic compounds, which are an environmentally undesirable by-product of paper mills. Such uses are described in, for example, Current opinion in Biotechnology 3: 261-266, 1992; J. Biotechnol. 25: 333-339, 1992; Hiroi et al., *Svensk papperstidning* 5: 162-166, 1976.
15 Since the environment in a paper mill is typically alkaline, the present laccase is more useful for this purpose than other known laccases, which function best under acidic conditions.

 Oxidation of dyes and other chromophoric compounds
20 leads to decolorization of the compounds. Laccase can be used for this purpose, which can be particularly advantageous in a situation in which a dye transfer between fabrics is undesirable, e.g., in the textile industry and in the detergent industry. Methods for dye transfer inhibition
25 and dye oxidation can be found in WO 92/01406, WO 92/18683, EP 0495836 and Calvo, *Mededelingen van de Faculteit Landbouw-wetenschappen/Rijksuniversitet Gent*.56: 1565-1567, 1991.

 The present laccase can also be used for the
30 polymerization of phenolic compounds present in liquids. An example of such utility is the treatment of juices, such as apple juice, so that the laccase will accelerate a

precipitation of the phenolic compounds present in the juice, thereby producing a more stable juice. Such applications have been described in Stutz, Fruit processing 7/93, 248-252, 1993; Maier et al., Dt. Lebensmittel-
5 rindschau 86(5): 137-142, 1990; Dietrich et al., Fluss. Obst 57(2): 67-73, 1990. The invention is further illustrated by the following non-limiting examples.

EXAMPLES

1. Purification and characterization of *R. solani* laccase

- 10 Individual isolates of *R. solani* cultured on potato dextrose agar (Difco) are examined for laccase enzyme formation by transferring a small piece of agar containing vigorous growth to 100 ml CFM (24.0 g potato dextrose broth, 3.0 g yeast extract, 1.0 ml Microelement solution
15 [0.80 g KH_2PO_4 , 0.64 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.11 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.80 g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.15 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, distilled water to 1000 ml], distilled water to 1000 ml) in a 500 ml shake flask. Incubation is at room temperature, at 200 rpm on an orbital shaker.
- 20 Samples are harvested at 50, 74, 122 and 170 hours, centrifuged and the clear supernatant analyzed for laccase with its ABTS (ABTS= 2,2'-azinobis (3 ethylbenzothiazoline-6-sulfonic acid). The analysis is carried out by adding 200 μl of 2mM ABTS in 0.1 M phosphate buffer, pH 7, and
25 observing the change in absorbance at 418 nm after 30 minutes incubation at room temperature (approximately 23-25° C). This method is modified from a peroxidase analysis method described by Pütter and Becker (Peroxidases, in: Bergmeyer, H.U.(ed.), Methods of Enzymatic Analysis, 3rd
30 ed., Vol.III, pp.286-293, 1983)

Each of the laccases harvested at 172 hours is electrophoretically separated and stained with ABTS as

chromogen. Several distinct patterns emerge; the strain RS 22 is shown to produce a laccase having a basic pI, and is chosen for further characterization.

Laccase activity is also determinable by the
5 syringaldazine method. Laccase catalyzes the oxidation of syringaldazine to tetramethoxy azo bis-methylene quinone under aerobic conditions, with a change of color from yellow to violet. 3000 μ l of 25 mM acetate buffer (containing 10mg/l cuprisulfate, 5 H₂O) at pH 5.5, 30°C, is mixed in a 1
10 cm cuvette with 225 μ l 0.28 mM syringaldazine (5mg solubilized in 25 ml ethanol and adjusted to 50 ml with demineralized water). The mixture is then mixed with 100 μ l of a laccase dilution (diluted in acetate buffer so that the increase in absorbance(Δ OD) is within the range of 0.1-0.6).
15 The reaction mixture is placed in a 30°C thermostated spectrophotometer and the reaction is followed at 530 nm for 10 to 70 seconds from the addition of laccase. The activity of the enzyme is calculated as Δ OD/minute x 0.677 x dilution factor, and is expressed as LACU.

20 For purification of the *Rhizoctonia* laccase, 2.1 liter of culture medium with a LACU activity of 0.19 LACU/ml is filtered through a 10 μ m filter and concentrated to 230 ml by ultrafiltration using a Filtron Minisette OMEGA membrane with a cutoff value of 10 kDa. The pH of the sample is 5.3
25 and the activity of the concentrated sample is determined to be 3.34 LACU/ml.

After pH adjustment to 4.5 and filtration due to slight precipitation, the sample is applied to a 40 ml S Sepharose Fast Flow column equilibrated with 20mM acetate buffer at pH
30 4.5 (buffer A). The column is washed in buffer A and eluted with buffer A containing 1 M NaCl. Active fractions are collected and pooled. This active pool is concentrated and

buffer exchanged to buffer A using an Amicon ultrafiltration unit equipped with a Diaflo YM10 membrane. This sample is rechromatographed on a 5 ml S Sepharose High Performance column using the method described above except that elution
 5 is carried out with a linear gradient over 30 column volumes from buffer A to buffer A containing 0.5 M NaCl. The fractions from this purification exhibiting highest activity are pooled. Approximately 45 mg laccase are obtained, when protein concentration is estimated by one absorption unit at
 10 A280 nm equal to 1mg/ml. The protein is >90% pure as judged by SDS-PAGE. The molecular weight estimated by SDS-PAGE is approximately 67 kDa. The specific activity of the purified protein is 1 LACU/mg. The pH profile of the purified protein, using syringaldazine as substrate is show in Table
 15 1, below.

Table 1.

	pH	5	6	7	8
20	% activity	0.5	31	100	59

For sequencing of the protein, peptides are generated using wither a lysine-specific protease from *Achromobacter* (*Achromobacter* protease I) or a glutamic acid specific
 25 protease from *Bacillus licheniformes*. The peptides are purified by reverse phase HPLC employing linear gradients of 80% 2-propanol containing 0.08% aqueous TFA (solvent B) in 0.1% aqueous TFA (solvent A).

N-terminal amino acid sequence analysis of the intact
 30 protein and of purified peptides are carried out in an Applied Biosystems 473A protein sequencer according to the manufacturer's instructions. Initial partial sequencing of

the isolated protein yields the following N-terminal sequence:

AVRNYKFDIKNVNVAPDGFQRPIVSV (SEQ. ID. NO.: 5)

The protein is then digested with either a lysine- or glutamic-acid specific protease, and following additional peptides identified. Peptides 1-4 can be aligned with sequences in the laccase of *Coriolus hirsutus*:

Peptide 1:

SQYVDGLRGPLVIYDPDDDH (SEQ. ID. NO: 6)

10 Peptide 2:

GLALVFAEAPSQIRQGVQSVQPDDA (SEQ. ID. NO.: 7)

Peptide 3:

SRYBVBBASTVVMLEBWYHTPAXVLE (SEQ. ID. NO. 8)

Peptide 4:

15 SLGPTPNYVNPXIRDVVRVGGTTVV (SEQ. ID. NO. 9)

Peptide 5:

IRYVGGBAVX(N?)RSVI (SEQ. ID. NO.: 10)

Peptide 6:

ILANPA (SEQ. ID. NO.: 11)

20 Peptide 7:

YEAPSLPT (SEQ. ID. NO.: 12)

An X in the above sequences designates an unidentified residue, and B represents a residue which is either aspartic acid or asparagine.

25

2. Isolation of *R. solani* laccase gene

A study of the known amino acid sequences of fungal laccases obtained from non-*Rhizoctonia* species (Choi et al., *supra*; German et al., *supra*; Saloheimo et al. *supra*; and 30 Kojima et al, *supra*) is conducted to determine the presence of consensus sequences among them. Two regions of high identity, IHWHGFFQ and TFWYHSH, are found near the amino

terminal third of the protein. Based on these consensus sequences and the corresponding DNA sequences, three degenerate oligonucleotides, O-lac2
[TGG/AAAGACCATA/GGTGTCTG/AGTA/G], its complement O-lac2r, and
5 O-lac3[ATCCAT/CTGGCAT/CGGG/CA/TTCTTCCAG/A], are synthesized using an Applied Biosystems 394 DNA/RNA synthesizer.

The synthesized oligos are used in a polymerase chain reaction (PCR) to screen *Rhizoctonia solani* genomic DNA for a laccase gene or fragment thereof. For amplifications of
10 genomic DNA, 0.5 µg of genomic DNA is incubated with 1µM of each primer, 200µM of dNTPs, and 1 U taq polymerase (Boehringer Mannheim) in [10 mM Tris-Cl, 1.5 mM MgCl₂, 50 mM KCl, 1 mg/ml gelatine; pH 8.3]. The reactions are incubated for 1x5 minutes at 95°C, 30x[1 minute at 95°C, 1 minute at
15 50-60°C, 1 minute at 72°C], and 1x5 minutes at 72°C. The PCR reactions amplify a DNA fragment of 220 nucleotides. The PCR product is cloned, according to manufacturer's directions, into the TA cloning vector (InVitrogen Corp.). Characterization of the PCR product by DNA sequencing of
20 individual clones distinguishes two separate laccase genes designated RSlac1 and RSlac2.

To prepare a *R. solani* genomic library, *R. solani* DNA is partially digested with restriction enzyme Sau3A, and electrophoresed through a 0.8% Sea Plaque Agarose (FMC
25 Bioproducts) in a Tris/Acetate/EDTA buffer to isolate those DNA fragments between 8.0 and 21 kb in size. The gel fractionated fragments are further purified with Beta-Agarase (New England Biolabs) according to manufacturer's instruction, and then ligated to lambda phage EMBL3 arms
30 with BamHI ends. The resulting phages are packaged in vitro using Gigapack II packaging extract (Stratagene). 25 ml of TB media+0.2% maltose and 10 MgSO₄ is inoculated into a 50 µl

aliquot of an overnight culture of *E. coli* K802 (supE, hsdR, gal, metB) and incubated at 37°C with shaking until the A600=0.5. 25 µl of a 1:10 and 1:50 dilution of the packaged phage are mixed with 250 µl of the K802 cells, and incubated
5 for 20 minutes at 37°C. To each dilution, 5 µl of melted top agar at 48°C are added. The mix is then plated onto prewarmed LB plates and incubated at 37°C for at least 12 hours. From these phage, a library of 170,000 plaques in *E. coli* K802 is created and amplified 100-fold for future
10 use.

To screen for the laccase gene, 25,000 plaques from the amplified genomic library are plated onto NZY/agarose plates for plaque lifts using conventional methods. Filters are screened using the 220 nucleotide PCR fragment randomly
15 labelled to 5×10^8 cpm/µg as a probe. Filters are hybridized in 50% formamide, 6xSSC for 16 hours at 42°C and washed with 0.5xSSC, 0.1% SDS at 65°C. Positive clones are picked and rescreened using conventional methods. The nine positive clones identified fell into two classes that by DNA sequence
20 analysis are shown to code for two different laccase genes, RSlac1 and RSlac2. The complete nucleotide sequence of each of these genes is determined using fluorescent nucleotides and an Applied Biosystems automatic DNA sequencer (Model 363A, version 1.2.0). The nucleotide and predicted amino
25 acid sequences are depicted in Figures 1 and 2.

For isolation of RSlac3, poly A RNA purified from *R. solani* mycelia grown in the presence of 1 mM anisidine is used as a template for cDNA synthesis using standard protocols. The cDNA is fractionated by electrophoresis
30 through a 0.8% agarose gel and DNA fragments between 1.7 and 3.5 kb in size are collected. A library is then created by cloning the size-fractionated cDNA into the yeast expression

vector pYES2. 3000 yeast transformants from this library are plated initially on YNB (1.7 g yeast nitrogen base without amino acids, 5 g $(\text{NH}_4)_2\text{SO}_4$ per liter) with 2% glucose. After 4 days growth at 30°C, the resulting colonies are replica plated to YNB with 0.1% glucose, 2% galactose and 2mM ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid; Sigma # A-1888). After 24 hours of growth at 30°C a single colony has a light green halo which gradually turns a dark purple. The plasmid from this colony is isolated and the insert sequenced. The sequence of the translated portion of the RSlac3 gene and protein is shown in SEQ.ID NOS. 13 and 14, and in Figure 4.

3. Expression of laccase gene

The plasmid pMWR-1 is a pUC derived vector containing the TAKA amylase transcription regulation signals and the TAKA amylase signal sequence. This plasmid is engineered with a unique SfiI site at the signal sequence cleavage site, and a 3' adjacent NsiI site such that these two restriction enzymes can be used to introduce, in frame, a foreign protein. Using a PCR reaction (conducted as described above, but with 100 ng of the appropriate linearized plasmid DNA as a template) and mutagenized primers, an SfiI site is introduced at amino acid 12 and amino acid 14 of RSlac1 and RSlac2, respectively, such that the protein coding sequences are in frame with the TAKA signal sequence. In addition, a PCR amplification is also used to introduce a PstI site (CTGCAG) at the 3' end of RSlac1 and an NsiI site (ATGCAT) at the 3' end of RSlac2.

To prepare for transformation, cells of *Aspergillus oryzae* are cultivated in YPG (1g/l yeast extract, 0.25 g K_2PO_4 , 0.125 g/ MgSO_4 , 3.75 g glucose) at 34°C with 100-120rpm

for 16-20 hours, then collected by filtration with miracloth. Cells are washed with Mg solution (0.6M $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), then 2-6 g of cells are taken up in 10 ml MgP (1.2M $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 10mM $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$; pH 5.8). To this is

5 added 1 ml of Novozyme® 234 (120 mg/ml MgP), and the sample kept on ice for 5 minutes. One ml of BSA (12 mg/ml) is added, and the sample shaken gently at 34-37°C. Protoplasts are collected by filtration through miracloth, and overlain with 5 ml of ST (0.6 M Sorbitol, 100mM Tris; pH 7). The

10 sample is spun at 2500 rpm for 15 minutes, and a band of protoplasts collected. Two volumes of STC (1.2M Sorbitol, 10 mM tris, 10 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$; pH 7.5) are added and the sample is spun at 2500 rpm for 5 minutes. The precipitate is washed twice with 5 ml of STC, and the protoplasts suspended in

15 0.5-1ml of STC.

For the transformation process, the protoplast concentration is adjusted to $1-5 \times 10^7/\text{ml}$. To 100 μl of protoplast solution is added a maximum of 10 μl of DNA solution (5-10 μg of supercoiled DNA) and 0.2 ml of PEG

20 (60% PEG4000, 10mM Tris, 10mM $\text{CaCl}_2 \cdot \text{H}_2\text{O}$; pH 7.5), and the combination is mixed well. The sample is kept at room temperature for 25 minutes; then to it is added first 0.2 ml PEG, with mixing, the 0.85 ml PEG with mixing. The mixture is kept at room temperature for 20 minutes, then spun at

25 4000 rpm for 15 minutes. The precipitate is washed with 2 ml of STC by spinning at 2500 rpm for 10 minutes. The protoplasts are resuspended in 0.2-0.5 ml of STC, and then spread on COVE plates. COVE medium (pH 7) contains 342.3 g/l sucrose, 25 g/l agar and a salt solution comprising 26 g/l

30 KCl, 26 g/l $\text{MgSO}_4 \cdot \text{H}_2\text{O}$, 76 g/l KH_2PO_4 , and 50 ml/l of trace metals; the trace metals are 40 mg/l $\text{NaB}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$, 400 mg/l

CuSO₄·5H₂O, 1200mg/l FeSO₄·7H₂O, 700mg/l MnSO₄·H₂O, 800mg/l Na₂MoO₂·2H₂O, 10 g/l ZnSO₄·7H₂O). After autoclaving, 10 ml/l of 1M filtrated acetamide and 5-10 ml of 3M CsCl are added to the solution. Transformants are selected by growth cells
5 on COVE medium which contains acetamide as the carbon source.

The confirmation of laccase production in the samples is determined by the ABTS oxidation method as described above on Cove medium with 2 mM ABTS, at pH 5 and 7.3. Both
10 RSlac1 and RSlac2 express laccase activity at pH 5 and pH 7, in contrast with a control laccase which shows substantially no activity at pH 7.3.

The products of the expression of each of RSlac1 and RSlac2 are tested for oxidase activity at various pHs using
15 syringaldazine as the substrate. The assay is conducted substantially as described above for the assay of the native protein, over pH range of 4-9. As shown in Figures 5 and 6, both laccases are active at pHs over pH 5, and RSlac1 has particularly good activity at pHs over 6. The pattern of
20 activity is generally comparable to that observed for the RSlac3 laccase isolated from RS 22 (see Table 1 above), with RSlac1 exhibiting the broadest range of activity.

Deposit of Biological Materials

The following biological materials have been deposited
25 under the terms of the Budapest Treaty in the International Mycological Institute, Genetic Resource Reference Collection, located at Bakeham Lane, Egham, Surrey TW20 9TY and given the following accession number.

30 <u>Deposit</u>	<u>Accession Number</u>
<i>Rhizoctonia solani</i> RS22	IMI CC 358730

The following biological materials have been deposited under the terms of the Budapest Treaty with the Agricultural Research Service Patent Culture Collection, Northern Regional Research Center, 1815 University Street, Peoria, Illinois, 61604 and given the following accession numbers.

DepositAccession Number

E. coli containing RSlac1 fused to
an α -amylase signal sequence

NRRL B-21141

(EMCC 00844)

10

E. coli containing RSlac2 with an
SfiI site insert

NRRL B-21142

(EMCC 00845)

15 *E. coli* containing RSlac3

NRRL B-21156

(EMCC 0088)

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Novo Nordisk A/S
(B) STREET: Novo Alle
(C) CITY: Bagsværd
(D) COUNTRY: Denmark
(E) POSTAL CODE (ZIP): DK-2880
(F) TELEPHONE: +45 4444 3333
(G) TELEFAX: +45 4449 3256
(F) TELEX: 37304

(i) APPLICANT:

(A) NAME: Novo Nordisk Biotech, Inc.
(B) STREET: 1445 Drew Avenue
(C) CITY: Davis, California
(D) COUNTRY: United States of America
(E) POSTAL CODE (ZIP): 95616-4880
(F) TELEPHONE: (916) 757-8100
(G) TELEFAX: (916) 758-0317

(ii) TITLE OF INVENTION: PURIFIED PH NEUTRAL LACCASES AND NUCLEIC ACIDS ENCODING SAME

(iii) NUMBER OF SEQUENCES: 14

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Novo Nordisk of North America, Inc.
(B) STREET: 405 Lexington Avenue, Suite 6400
(C) CITY: New York
(D) STATE: New York
(E) COUNTRY: USA
(F) ZIP: 10174-6401

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: to be assigned
(B) FILING DATE: 13-SEP-1994

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/172,331
(B) FILING DATE: 22-DEC-1993

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/122,230
(B) FILING DATE: 17-SEP-1993

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/122,827
(B) FILING DATE: 17-SEP-1993

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/162,827
(B) FILING DATE: 03-DEC-1993

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Lowney Dr., Karen A.
(B) REGISTRATION NUMBER: 31,274
(C) REFERENCE/DOCKET NUMBER: 4052.204-WO

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 212-867-0123

(B) TELEFAX: 212-878-9655

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2838 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Rhizoctonia laccase

(ix) FEATURE:

(A) NAME/KEY: intron

(B) LOCATION: 302..351

(ix) FEATURE:

(A) NAME/KEY: intron

(B) LOCATION: 463..512

(ix) FEATURE:

(A) NAME/KEY: intron

(B) LOCATION: 576..633

(ix) FEATURE:

(A) NAME/KEY: intron

(B) LOCATION: 760..818

(ix) FEATURE:

(A) NAME/KEY: intron

(B) LOCATION: 822..877

(ix) FEATURE:

(A) NAME/KEY: intron

(B) LOCATION: 1001..1054

(ix) FEATURE:

(A) NAME/KEY: intron

(B) LOCATION: 1316..1372

(ix) FEATURE:

(A) NAME/KEY: intron

(B) LOCATION: 1697..1754

(ix) FEATURE:

(A) NAME/KEY: intron

(B) LOCATION: 1827..1880

(ix) FEATURE:

(A) NAME/KEY: intron

(B) LOCATION: 1992..2051

(ix) FEATURE:

(A) NAME/KEY: intron

(B) LOCATION: 2157..2206

(ix) FEATURE:

(A) NAME/KEY: intron

(B) LOCATION: 2348..2404

(ix) FEATURE:

(A) NAME/KEY: intron
(B) LOCATION: 2438..2498

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: join(170..301, 352..462, 513..575, 634..759, 819
..821, 878..1000, 1055..1315, 1373..1696, 1755
..1826, 1881..1991, 2052..2156, 2207..2347, 2405
..2437, 2499..2621)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

AGCGTCACAC CAGACATCGG ATGAAAACGG AAAGTGTATG CGCCATTGGA CGTCTGCGGC      60
AACCACGTGT CATCTCGCGA GCTAACATGG GCGACGTATA AGAAGAACGC GAGAATGGGC      120
AGATTTTCGAT ATCCCCTCTC GTCTCGGTTT TGGTCTCGGC TTGCCTCTA ATG GCG      175
                               Met Ala
                               1

CGC ACC ACT TTC CTT GTC TCG GTT TCG CTC TTT GTT TCC GCT GTT CTT      223
Arg Thr Thr Phe Leu Val Ser Val Ser Leu Phe Val Ser Ala Val Leu
      5                               10                               15

GCG CGC ACC GTC GAG TAC GGC TTG AAG ATT AGT GAT GGG GAG ATA GCT      271
Ala Arg Thr Val Glu Tyr Gly Leu Lys Ile Ser Asp Gly Glu Ile Ala
      20                               25                               30

CCT GAC GGT GTT AAG CGT AAT GCG ACT TTG GTACGCACTC CTTGTAATCC      321
Pro Asp Gly Val Lys Arg Asn Ala Thr Leu
      35                               40

AACAATTCAA GGTTCCTGAT GCTTGGTCAG GTA AAT GGA GGG TAT CCC GGT CCA      375
                               Val Asn Gly Gly Tyr Pro Gly Pro
                               45                               50

CTC ATT TTT GCC AAC AAG GGG GAT ACT CTC AAA GTC AAG GTC CAA AAC      423
Leu Ile Phe Ala Asn Lys Gly Asp Thr Leu Lys Val Lys Val Gln Asn
      55                               60                               65

AAG CTC ACG AAT CCT GAG ATG TAT CGC ACC ACT TCC ATC GTATGTTTCGT      472
Lys Leu Thr Asn Pro Glu Met Tyr Arg Thr Thr Ser Ile
      70                               75                               80

TCGATATCTA CTAATACATC CGTCGCTAAA TATCTTGTAG CAT TGG CAC GGT CTC      527
                               His Trp His Gly Leu
                               85

TTA CAA CAT AGA AAC GCC GAC GAC GAC GGT CCT TCG TTC GTC ACT CAG      575
Leu Gln His Arg Asn Ala Asp Asp Asp Gly Pro Ser Phe Val Thr Gln
      90                               95                               100

GTAGGATTCT GGAAGGTTGG CCTGAACTCT CTGTTAACCG ACAACCCGAT GTCACCAG      633

TGC CCG ATT GTT CCA CGC GAG TCG TAT ACT TAC ACC ATA CCT CTG GAC      681
Cys Pro Ile Val Pro Arg Glu Ser Tyr Thr Tyr Thr Ile Pro Leu Asp
      105                               110                               115

GAT CAA ACC GGA ACC TAT TGG TAC CAT AGC CAC TTG AGT TCG CAA TAC      729
Asp Gln Thr Gly Thr Tyr Trp Tyr His Ser His Leu Ser Ser Gln Tyr
      120                               125                               130

GTT GAT GGT CTT CGA GGC CCG CTG GTA ATC GTGAGTATCT TGACTTGTCT      779
Val Asp Gly Leu Arg Gly Pro Leu Val Ile
      135                               140

```


ACTGAAGGCA ACGAGACTAA AACAAGCGTC GATTCACAG TAT GTTCGTCTCC	831
Tyr 145	
CCTTTATTTA GCTCTGGATC TTCATTCTC ACGTAATACA TGATAG GAT CCC AAG	886
Asp Pro Lys	
GAT CCT CAC AGG CGT TTG TAT GAT GTT GAC GAT GAG AAG ACC GTC CTG	934
Asp Pro His Arg Arg Leu Tyr Asp Val Asp Asp Glu Lys Thr Val Leu	
150 155 160	
ATC ATC GGT GAC TGG TAT CAT GAA TCG TCC AAG GCA ATC CTT GCT TCT	982
Ile Ile Gly Asp Trp Tyr His Glu Ser Ser Lys Ala Ile Leu Ala Ser	
165 170 175 180	
GGT AAC ATT ACC CGA CAG GTAAGTGATA CATGCCGGTC CCAGAAAAT	1030
Gly Asn Ile Thr Arg Gln	
185	
TCTCTAAATT CATTTTAATT ACAG CGA CCG GTC TCT GCC ACC ATC AAC GGC	1081
Arg Pro Val Ser Ala Thr Ile Asn Gly	
190 195	
AAA GGT CGA TTT GAC CCT GAC AAC ACT CCT GCC AAC CCA GAT ACT CTG	1129
Lys Gly Arg Phe Asp Pro Asp Asn Thr Pro Ala Asn Pro Asp Thr Leu	
200 205 210	
TAC ACC CTC AAG GTC AAG CGA GGG AAG CGC TAT CGT CTG CGT GTC ATC	1177
Tyr Thr Leu Lys Val Lys Arg Gly Lys Arg Tyr Arg Leu Arg Val Ile	
215 220 225	
AAT AGC TCG GAG ATC GCT TCG TTC CGA TTC AGT GTG GAA GGT CAC AAG	1225
Asn Ser Ser Glu Ile Ala Ser Phe Arg Phe Ser Val Glu Gly His Lys	
230 235 240	
GTG ACT GTG ATT GCT GCC GAT GGC GTC TCT ACC AAA CCG TAT CAG GTC	1273
Val Thr Val Ile Ala Ala Asp Gly Val Ser Thr Lys Pro Tyr Gln Val	
245 250 255	
GAT GCG TTT GAT ATT CTA GCA GGA CAG CGC ATA GAT TGC GTC	1315
Asp Ala Phe Asp Ile Leu Ala Gly Gln Arg Ile Asp Cys Val	
260 265 270	
GTAAGTGTGCG TCCGAACCCA CATCTGAGCT CAAGTGTGTA TACATGCGCG CTTATAG	1372
GTG GAG GCG AAC CAA GAA CCC GAC ACA TAC TGG ATC AAC GCA CCG CTG	1420
Val Glu Ala Asn Gln Glu Pro Asp Thr Tyr Trp Ile Asn Ala Pro Leu	
275 280 285	
ACC AAC GTG CCC AAC AAG ACC GCT CAG GCT CTC CTC GTT TAT GAG GAG	1468
Thr Asn Val Pro Asn Lys Thr Ala Gln Ala Leu Leu Val Tyr Glu Glu	
290 295 300 305	
GAT CGT CGG CCG TAC CAC CCT CCA AAG GGC CCG TAT CGC AAG TGG AGC	1516
Asp Arg Arg Pro Tyr His Pro Pro Lys Gly Pro Tyr Arg Lys Trp Ser	
310 315 320	
GTC TCT GAG GCG ATC ATC AAG TAC TGG AAT CAC AAG CAC AAG CAC GGA	1564
Val Ser Glu Ala Ile Ile Lys Tyr Trp Asn His Lys His Lys His Gly	
325 330 335	
CGT GGT TTG CTG TCT GGA CAT GGA GGT CTC AAG GCT CGG ATG ATC GAG	1612
Arg Gly Leu Leu Ser Gly His Gly Gly Leu Lys Ala Arg Met Ile Glu	
340 345 350	
GGT AGC CAT CAT CTG CAT TCG CGC AGC GTC GTT AAG CGC CAG AAT GAG	1660

Gly Ser His His Leu His Ser Arg Ser Val Val Lys Arg Gln Asn Glu 355 360 365	
ACC ACC ACT GTT GTA ATG GAC GAG AGC AAG CTC GTT GTAAGTACCA Thr Thr Thr Val Val Met Asp Glu Ser Lys Leu Val 370 375 380	1706
TATTTAAAAG TTGGTTGGGT TTCGAATACT TATTTCAACT TTTCTTAG CCA CTG GAA Pro Leu Glu	1763
TAC CCC GGC GCT GCA TGC GGG TCT AAA CCT GCT GAC CTC GTC TTG GAT Tyr Pro Gly Ala Ala Cys Gly Ser Lys Pro Ala Asp Leu Val Leu Asp 385 390 395 400	1811
CTC ACT TTT GGT TTG GTATGTAGCC AAATCGCCCA TATACAGGAT ACTGAATATT Leu Thr Phe Gly Leu 405	1866
GT TTGTGCGT GTAG AAC TTT GCT ACC GGG CAC TGG ATG ATC AAC GGT ATC Asn Phe Ala Thr Gly His Trp Met Ile Asn Gly Ile 410 415	1916
CCA TAC GAG TCT CCC AAA ATC CCC ACA TTG CTC AAG ATC CTC ACT GAT Pro Tyr Glu Ser Pro Lys Ile Pro Thr Leu Leu Lys Ile Leu Thr Asp 420 425 430	1964
GAG GAC GGG GTT ACC GAG TCT GAC TTC GTATGTTCCC TTTTCGGTAT Glu Asp Gly Val Thr Glu Ser Asp Phe 435 440	2011
CTTCGTATGC GTGCACTGAC TCGTGCTGGT GGAATTTAG ACC AAG GAG GAG CAC Thr Lys Glu Glu His 445	2066
ACA GTC ATA CTC CCG AAG AAC AAA TGC ATC GAA TTC AAC ATC AAG GGG Thr Val Ile Leu Pro Lys Asn Lys Cys Ile Glu Phe Asn Ile Lys Gly 450 455 460	2114
AAC TCG GGT ATT CCC ATT ACG CAC CCC GTA CAT CTT CAC GGT Asn Ser Gly Ile Pro Ile Thr His Pro Val His Leu His Gly 465 470 475	2156
GTAAGTGCAT ATCGGATGGT TTACGATACT AAGGCTCATC AACTTTTTAG CAC ACT His Thr	2212
TGG GAT GTC GTA CAA TTT GGC AAC AAC CCA CCC AAT TAT GTC AAT CCT Trp Asp Val Val Gln Phe Gly Asn Asn Pro Pro Asn Tyr Val Asn Pro 480 485 490 495	2260
CCC CGT AGG GAC GTG GTT GGC TCT ACA GAT GCG GGT GTG AGG ATT CAG Pro Arg Arg Asp Val Val Gly Ser Thr Asp Ala Gly Val Arg Ile Gln 500 505 510	2308
TTC AAG ACC GAC AAT CCA GGA CCG TGG TTC CTG CAC TGC GTGCGTCGGT Phe Lys Thr Asp Asn Pro Gly Pro Trp Phe Leu His Cys 515 520	2357
CCCCATCGTC CGTTATGGTT TTTCTAATAC GTCCCATCTT ATTTTAG CAT ATT GAC His Ile Asp 525	2413
TGG CAT CTT GAG GAG GGT TTC GCA GTGAGTACTG AGACCTAAGT GCTACTCGGC Trp His Leu Glu Glu Gly Phe Ala 530 535	2467

TCATTACTGA TTACCGCATG TATGCGTCTA G ATG GTG TTT GCT GAA GCG CCC 2519
Met Val Phe Ala Glu Ala Pro
540

GAA GCC GTC AAG GGA GGT CCA AAG AGC GTG GCC GTG GAC TCT CAG TGG 2567
Glu Ala Val Lys Gly Gly Pro Lys Ser Val Ala Val Asp Ser Gln Trp
545 550 555

GAA GGG CTG TGT GGC AAG TAC GAC AAC TGG CTA AAA TCA AAT CCG GGC 2615
Glu Gly Leu Cys Gly Lys Tyr Asp Asn Trp Leu Lys Ser Asn Pro Gly
560 565 570

CAG CTG TAGGCGTATC GCAGCCACAT TGGTGATGAT TGAAAGTTGC ATCTTGTTCC 2671
Gln Leu
575

TATAACCGGC TCTTATATAC GGGTGTCTCC CAGTAAAGTC GTAGCCCAAT TTCAGCCGAG 2731

ACAGATATTT AGTGGACTCT TACTCTTG TG TCCCATTTGAC GCACATCGTT GCATCAAACC 2791

TGCTTTTTTAT CGTCCCTCTT TGTAATTTGT GTTGCTGTAA TGTATCG 2838

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 576 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Arg Thr Thr Phe Leu Val Ser Val Ser Leu Phe Val Ser Ala
1 5 10 15

Val Leu Ala Arg Thr Val Glu Tyr Gly Leu Lys Ile Ser Asp Gly Glu
20 25 30

Ile Ala Pro Asp Gly Val Lys Arg Asn Ala Thr Leu Val Asn Gly Gly
35 40 45

Tyr Pro Gly Pro Leu Ile Phe Ala Asn Lys Gly Asp Thr Leu Lys Val
50 55 60

Lys Val Gln Asn Lys Leu Thr Asn Pro Glu Met Tyr Arg Thr Thr Ser
65 70 75 80

Ile His Trp His Gly Leu Leu Gln His Arg Asn Ala Asp Asp Asp Gly
85 90 95

Pro Ser Phe Val Thr Gln Cys Pro Ile Val Pro Arg Glu Ser Tyr Thr
100 105 110

Tyr Thr Ile Pro Leu Asp Asp Gln Thr Gly Thr Tyr Trp Tyr His Ser
115 120 125

His Leu Ser Ser Gln Tyr Val Asp Gly Leu Arg Gly Pro Leu Val Ile
130 135 140

Tyr Asp Pro Lys Asp Pro His Arg Arg Leu Tyr Asp Val Asp Asp Glu
145 150 155 160

Lys Thr Val Leu Ile Ile Gly Asp Trp Tyr His Glu Ser Ser Lys Ala
165 170 175

Ile Leu Ala Ser Gly Asn Ile Thr Arg Gln Arg Pro Val Ser Ala Thr
 180 185 190
 Ile Asn Gly Lys Gly Arg Phe Asp Pro Asp Asn Thr Pro Ala Asn Pro
 195 200 205
 Asp Thr Leu Tyr Thr Leu Lys Val Lys Arg Gly Lys Arg Tyr Arg Leu
 210 215 220
 Arg Val Ile Asn Ser Ser Glu Ile Ala Ser Phe Arg Phe Ser Val Glu
 225 230 235 240
 Gly His Lys Val Thr Val Ile Ala Ala Asp Gly Val Ser Thr Lys Pro
 245 250 255
 Tyr Gln Val Asp Ala Phe Asp Ile Leu Ala Gly Gln Arg Ile Asp Cys
 260 265 270
 Val Val Glu Ala Asn Gln Glu Pro Asp Thr Tyr Trp Ile Asn Ala Pro
 275 280 285
 Leu Thr Asn Val Pro Asn Lys Thr Ala Gln Ala Leu Leu Val Tyr Glu
 290 295 300
 Glu Asp Arg Arg Pro Tyr His Pro Pro Lys Gly Pro Tyr Arg Lys Trp
 305 310 315 320
 Ser Val Ser Glu Ala Ile Ile Lys Tyr Trp Asn His Lys His Lys His
 325 330 335
 Gly Arg Gly Leu Leu Ser Gly His Gly Gly Leu Lys Ala Arg Met Ile
 340 345 350
 Glu Gly Ser His His Leu His Ser Arg Ser Val Val Lys Arg Gln Asn
 355 360 365
 Glu Thr Thr Thr Val Val Met Asp Glu Ser Lys Leu Val Pro Leu Glu
 370 375 380
 Tyr Pro Gly Ala Ala Cys Gly Ser Lys Pro Ala Asp Leu Val Leu Asp
 385 390 395 400
 Leu Thr Phe Gly Leu Asn Phe Ala Thr Gly His Trp Met Ile Asn Gly
 405 410 415
 Ile Pro Tyr Glu Ser Pro Lys Ile Pro Thr Leu Leu Lys Ile Leu Thr
 420 425 430
 Asp Glu Asp Gly Val Thr Glu Ser Asp Phe Thr Lys Glu Glu His Thr
 435 440 445
 Val Ile Leu Pro Lys Asn Lys Cys Ile Glu Phe Asn Ile Lys Gly Asn
 450 455 460
 Ser Gly Ile Pro Ile Thr His Pro Val His Leu His Gly His Thr Trp
 465 470 475 480
 Asp Val Val Gln Phe Gly Asn Asn Pro Pro Asn Tyr Val Asn Pro Pro
 485 490 495
 Arg Arg Asp Val Val Gly Ser Thr Asp Ala Gly Val Arg Ile Gln Phe
 500 505 510
 Lys Thr Asp Asn Pro Gly Pro Trp Phe Leu His Cys His Ile Asp Trp
 515 520 525
 His Leu Glu Glu Gly Phe Ala Met Val Phe Ala Glu Ala Pro Glu Ala

530

535

540

Val Lys Gly Gly Pro Lys Ser Val Ala Val Asp Ser Gln Trp Glu Gly
 545 550 555 560

Leu Cys Gly Lys Tyr Asp Asn Trp Leu Lys Ser Asn Pro Gly Gln Leu
 565 570 575

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3117 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Rhizoctonia laccase

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: join(393..524, 577..687, 737..799, 860..985, 1043
 ..1045, 1097..1219, 1269..1538, 1601..1996, 2047
 ..2118, 2174..2284, 2338..2439, 2495..2635, 2693
 ..2725, 2786..2899)

(ix) FEATURE:

- (A) NAME/KEY: intron
- (B) LOCATION: 525..576

(ix) FEATURE:

- (A) NAME/KEY: intron
- (B) LOCATION: 688..736

(ix) FEATURE:

- (A) NAME/KEY: intron
- (B) LOCATION: 800..859

(ix) FEATURE:

- (A) NAME/KEY: intron
- (B) LOCATION: 986..1042

(ix) FEATURE:

- (A) NAME/KEY: intron
- (B) LOCATION: 1220..1268

(ix) FEATURE:

- (A) NAME/KEY: intron
- (B) LOCATION: 1539..1600

(ix) FEATURE:

- (A) NAME/KEY: intron
- (B) LOCATION: 1823..1936

(ix) FEATURE:

- (A) NAME/KEY: intron
- (B) LOCATION: 1973..2046

(ix) FEATURE:

- (A) NAME/KEY: intron
- (B) LOCATION: 2119..2173

(ix) FEATURE:

- (A) NAME/KEY: intron

(B) LOCATION: 2285..2337

(ix) FEATURE:

(A) NAME/KEY: intron

(B) LOCATION: 2440..2494

(ix) FEATURE:

(A) NAME/KEY: intron

(B) LOCATION: 2636..2692

(ix) FEATURE:

(A) NAME/KEY: intron

(B) LOCATION: 1046..1096

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAGTGATCCG CCAGAGTTCA GCGCGATAAG TTCCTAAATA GTCATTGCGC TATTCGTGTA	60
CCTCAGCATA CTGACGACAT ACCGCCAGAT CGCCCTCGGT TCGGGCGTGG CATACGTTCTG	120
CAAGGGCACC TCACGGAGCA AACTCTAAAA AGCTTCGGCA TGGATTGCAT TTTGTATTGT	180
AAACAAGTTA CGAGAAAAAC AATAGATCAG TTTTGTCCGA ATCGGATGGC TTGAAACGGA	240
AGTACCGATG GCCGATCCGA GTCGAATGAA TTAACGCATC TGAAACGGGA CCCTGAGTCG	300
AGGCACCCGC CGGCCTTGGC CGTATAAGTC ACTTGTCGCC AACTAGCACT TTTTCATTCC	360
CCCTTTTCTT CTTCCTCGTC TTCTTCTTCT CT ATG GCT CGG TCG ACT ACT TCA	413
Met Ala Arg Ser Thr Thr Ser	
1 5	
CTC TTT GCA CTG TCT CTC GTT GCT TCA GCG TTT GCT CGA GTC GTT GAC	461
Leu Phe Ala Leu Ser Leu Val Ala Ser Ala Phe Ala Arg Val Val Asp	
10 15 20	
TAT GGG TTT GAT GTG GCT AAT GGG GCA GTT GCT CCG GAT GGT GTA ACA	509
Tyr Gly Phe Asp Val Ala Asn Gly Ala Val Ala Pro Asp Gly Val Thr	
25 30 35	
AGG AAC GCG GTT CTC GTGAGTTAGC TGTAAGATGG TGTATATGCT GGTTCCTAA	564
Arg Asn Ala Val Leu	
40	
CGGGAATGTC AG GTC AAT GGT CGC TTC CCT GGT CCA TTG ATC ACC GCC	612
Val Asn Gly Arg Phe Pro Gly Pro Leu Ile Thr Ala	
45 50 55	
AAC AAG GGG GAT ACA CTT AAA ATC ACC GTG CGG AAT AAA CTC TCC GAT	660
Asn Lys Gly Asp Thr Leu Lys Ile Thr Val Arg Asn Lys Leu Ser Asp	
60 65 70	
CCA ACT ATG CGA AGG AGC ACG ACC ATC GTTAGTACTT CCCCTCATCT	707
Pro Thr Met Arg Arg Ser Thr Thr Ile	
75 80	
GTCTTGAAAC TTTCTCATCT TTTTGAAG CAC TGG CAC GGT CTG CTC CAA CAC	760
His Trp His Gly Leu Leu Gln His	
85	
AGG ACG GCA GAA GAA GAT GGC CCG GCC TTT GTA ACC CAG GTATGCCTTA	809
Arg Thr Ala Glu Glu Asp Gly Pro Ala Phe Val Thr Gln	
90 95 100	
TCCTATCGCT GCTCTGTCCC CGCGTCCTTC CCTGACTCGG GCGATTCTAG TGC CCG	865
Cys Pro	

ATT CCT CCG CAA GAA TCG TAC ACC TAT ACG ATG CCG CTC GGC GAA CAG 913
 Ile Pro Pro Gln Glu Ser Tyr Thr Tyr Thr Met Pro Leu Gly Glu Gln
 105 110 115 120

ACC GGC ACG TAT TGG TAC CAC AGC CAC TTG AGC TCC CAG TAT GTG GAC 961
 Thr Gly Thr Tyr Trp Tyr His Ser His Leu Ser Ser Gln Tyr Val Asp
 125 130 135

CCG TTC CGT GCG CCC ATC GTT ATT GTAAGTCTTC ATTTAACCTT ATTCTTGCTT 1015
 Gly Leu Arg Gly Pro Ile Val Ile
 140

ATGGCTGATT GTGACGTCGT GGTAGT ATG TTCGTGGCTT CCACAAGAAG 1065
 Met
 145

TCAGCAGCCC TTGAAGCTAA CTTTATTCCA G GAC CCC CAC GAC CCG TAC AGA 1117
 Asp Pro His Asp Pro Tyr Arg
 150

AAC TAC TAT GAT GTC GAC GAC GAG CGT ACG GTC TTT ACT TTA GCA GAC 1165
 Asn Tyr Tyr Asp Val Asp Asp Glu Arg Thr Val Phe Thr Leu Ala Asp
 155 160 165

TGG TAC CAC ACG CCG TCG GAG GCT ATC ATT GCC ACC CAC GAT GTC TTG 1213
 Trp Tyr His Thr Pro Ser Glu Ala Ile Ile Ala Thr His Asp Val Leu
 170 175 180

AAA ACG GTACGCGTTA ATCCTTCTAG CTTTCTTTCC TTGGGTCACCT TTCTATCAG 1268
 Lys Thr
 185

ATC CCC GAC TCG GGT ACG ATC AAC GGC AAA GGC AAA TAC GAT CCT GCT 1316
 Ile Pro Asp Ser Gly Thr Ile Asn Gly Lys Gly Lys Tyr Asp Pro Ala
 190 195 200

TCG GCT AAC ACC AAC AAC ACG ACA CTC GAG AAC CTC TAC ACT CTC AAA 1364
 Ser Ala Asn Thr Asn Asn Thr Thr Leu Glu Asn Leu Tyr Thr Leu Lys
 205 210 215

GTC AAA CGC GGC AAG CGG TAT CGC CTG AGG ATT ATC AAC GCC TCG GCC 1412
 Val Lys Arg Gly Lys Arg Tyr Arg Leu Arg Ile Ile Asn Ala Ser Ala
 220 225 230

ATC GCT TCG TTC CGG TTC GGC GTG CAG GGC CAC AAG TGC ACG ATC ATC 1460
 Ile Ala Ser Phe Arg Phe Gly Val Gln Gly His Lys Cys Thr Ile Ile
 235 240 245 250

GAG GCT GAT GGC GTC CTC ACC AAA CCG ATC GAG GTC GAT GCG TTT GAT 1508
 Glu Ala Asp Gly Val Leu Thr Lys Pro Ile Glu Val Asp Ala Phe Asp
 255 260 265

ATT CTA GCA GGC CAG AGG TAT AGC TGC ATC GTAAGTCTAC CTATGCCTTG 1558
 Ile Leu Ala Gly Gln Arg Tyr Ser Cys Ile
 270 275

TTGTGGAGAT AAGAACCTGA CTGAATGTAT GCGCTCCAAT AG TTG AAG GCC GAC 1612
 Leu Lys Ala Asp
 280

CAA GAT CCT GAT TCC TAC TGG ATA AAT GCG CCA ATC ACA AAC GTT CTC 1660
 Gln Asp Pro Asp Ser Tyr Trp Ile Asn Ala Pro Ile Thr Asn Val Leu
 285 290 295

AAC ACC AAC GTC CAG GCA TTG CTA GTG TAT GAA GAT GAC AAG CGT CCT 1708

Asn	Thr	Asn	Val	Gln	Ala	Leu	Leu	Val	Tyr	Glu	Asp	Asp	Lys	Arg	Pro	
			300					305					310			
ACT	CAC	TAC	CCC	TGG	AAG	CCG	TTT	TTG	ACA	TGG	AAG	ATA	TCA	AAT	GAA	1756
Thr	His	Tyr	Pro	Trp	Lys	Pro	Phe	Leu	Thr	Trp	Lys	Ile	Ser	Asn	Glu	
		315					320					325				
ATC	ATT	CAG	TAC	TGG	CAG	CAC	AAG	CAC	GGG	TCG	CAC	GGT	CAC	AAG	GGA	1804
Ile	Ile	Gln	Tyr	Trp	Gln	His	Lys	His	Gly	Ser	His	Gly	His	Lys	Gly	
		330					335					340				
AAG	GGG	CAT	CAT	CAT	AAA	GTC	CGG	GCC	ATT	GGA	GGT	GTA	TCC	GGG	TTG	1852
Lys	Gly	His	His	His	Lys	Val	Arg	Ala	Ile	Gly	Gly	Val	Ser	Gly	Leu	
					350					355					360	
AGC	TCC	AGG	GTT	AAG	AGC	CGG	GCG	AGT	GAC	CTA	TCG	AAG	AAG	GCT	GTC	1900
Ser	Ser	Arg	Val	Lys	Ser	Arg	Ala	Ser	Asp	Leu	Ser	Lys	Lys	Ala	Val	
				365					370					375		
GAG	TTG	GCT	GCT	GCA	CTC	GTT	GCG	GGT	GAG	GCC	GAG	TTG	GAC	AAG	AGG	1948
Glu	Leu	Ala	Ala	Ala	Leu	Val	Ala	Gly	Glu	Ala	Glu	Leu	Asp	Lys	Arg	
			380					385					390			
CAG	AAT	GAG	GAT	AAT	TCG	ACT	ATT	GTA	TTG	GAT	GAG	ACC	AAG	CTT	ATT	1996
Gln	Asn	Glu	Asp	Asn	Ser	Thr	Ile	Val	Leu	Asp	Glu	Thr	Lys	Leu	Ile	
		395					400					405				
GTAAGTCCCT	TAATTTTTTTT	CGGTGTCACG	GAAGCTAACC	CGCGTAATAG	CCG	TTG										2052
						Pro	Leu								410	
GTT	CAA	CCT	GGT	GCA	CCG	GGC	GGC	TCC	AGA	CCA	GCT	GAC	GTC	GTG	GTC	2100
Val	Gln	Pro	Gly	Ala	Pro	Gly	Gly	Ser	Arg	Pro	Ala	Asp	Val	Val	Val	
				415					420					425		
CCT	CTG	GAC	TTT	GGC	CTC	GTATGTGGCT	TCTTGTTATT	CGTCCGGAAT								2148
Pro	Leu	Asp	Phe	Gly	Leu											
				430												
GCAAACCTGAT	TTGGGTGGGC	TATAG	AAC	TTT	GCC	AAC	GGA	CTG	TGG	ACG	ATA					2200
			Asn	Phe	Ala	Asn	Gly	Leu	Trp	Thr	Ile					
						435					440					
AAC	AAT	GTC	TCC	TAC	TCC	CCT	CCG	GAT	GTC	CCT	ACT	CTC	CTC	AAG	ATC	2248
Asn	Asn	Val	Ser	Tyr	Ser	Pro	Pro	Asp	Val	Pro	Thr	Leu	Leu	Lys	Ile	
			445					450					455			
TTG	ACC	GAC	AAA	GAC	AAA	GTC	GAC	GCT	TCT	GAC	TTC	GTAGGTTTCCT				2294
Leu	Thr	Asp	Lys	Asp	Lys	Val	Asp	Ala	Ser	Asp	Phe					
		460					465									
CTTCTTCTTT	TCAAACCTAGC	TACTGACATT	AAGTGAACGT	CAG	ACG	GCC	GAT	GAA								2349
					Thr	Ala	Asp	Glu								
						470										
CAC	ACG	TAT	ATT	CTT	CCA	AAG	AAC	CAA	GTT	GTC	GAG	TTG	CAC	ATC	AAG	2397
His	Thr	Tyr	Ile	Leu	Pro	Lys	Asn	Gln	Val	Val	Glu	Leu	His	Ile	Lys	
		475				480					485					
GGA	CAG	GCT	TTG	GGA	ATC	GTA	CAC	CCC	CTT	CAT	CTG	CAT	GGC			2439
Gly	Gln	Ala	Leu	Gly	Ile	Val	His	Pro	Leu	His	Leu	His	Gly			
		490			495				500							
GTACGTCTTT	CTCACACTGT	TCCAGCTCCT	ATTCTCTAAC	ACACTCCTGC	GATAG	CAT										2497
						His										

GCG TTC GAC GTC GTC CAA TTC GGC GAC AAC GCT CCA AAC TAC GTG AAC	2545
Ala Phe Asp Val Val Gln Phe Gly Asp Asn Ala Pro Asn Tyr Val Asn	
505 510 515 520	
CCT CCG CGT AGG GAT GTA GTA GGC GTA ACT GAT GCT GGA GTC CGT ATC	2593
Pro Pro Arg Arg Asp Val Val Gly Val Thr Asp Ala Gly Val Arg Ile	
525 530 535	
CAG TTC AGA ACC GAT AAC CCG GGC CCT TGG TTC CTC CAT TGC	2635
Gln Phe Arg Thr Asp Asn Pro Gly Pro Trp Phe Leu His Cys	
540 545 550	
GTATGCTCTT CATCTCCAC CGCTTGTCT TTAATTATGG TTTACCTTGC GATTTAG	2692
CAC ATT GAT TGG CAC TTG GAA GAA GGA TTT GCT GTAAGTTATT ATTCCTATTC	2745
His Ile Asp Trp His Leu Glu Glu Gly Phe Ala	
555 560	
CGAAGCATCG GGGAGATGCT AACCAAGGGT GTGTTTTAAG ATG GTA TTC GCC GAA	2800
Met Val Phe Ala Glu	
565	
GCG CCT GAA GAT ATC AAG AAA GGC TCT CAG AGT GTC AAG CCT GAC GGA	2848
Ala Pro Glu Asp Ile Lys Lys Gly Ser Gln Ser Val Lys Pro Asp Gly	
570 575 580	
CAA TGG AAG AAA CTA TGC GAG AAG TAT GAG AAG TTG CCT GAA GCA CTG	2896
Gln Trp Lys Lys Leu Cys Glu Lys Tyr Glu Lys Leu Pro Glu Ala Leu	
585 590 595	
CAG TGAAGTTGCA GTTGTTCCTT ATTCGGGAAC TGGCTCACTA TTCCTTTTGC	2949
Gln	
ATAATTCGGA CTTTTATTTT GGGACATTAT TGGACTATGG ACTTGTGTTGT CACACCCTCG	3009
CTCACTGTGT CCCTCGTTGA GTACCTATAC TCTATTCGTA TAGTGGGAAT ATGGAATATC	3069
GGATGTAATA AATGCTCGTG CGTTTGGTGC TCGAAATGGG GTAGGACT	3117

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 599 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Ala	Arg	Ser	Thr	Thr	Ser	Leu	Phe	Ala	Leu	Ser	Leu	Val	Ala	Ser
1				5					10					15	
Ala	Phe	Ala	Arg	Val	Val	Asp	Tyr	Gly	Phe	Asp	Val	Ala	Asn	Gly	Ala
		20						25					30		
Val	Ala	Pro	Asp	Gly	Val	Thr	Arg	Asn	Ala	Val	Leu	Val	Asn	Gly	Arg
		35					40					45			
Phe	Pro	Gly	Pro	Leu	Ile	Thr	Ala	Asn	Lys	Gly	Asp	Thr	Leu	Lys	Ile
	50					55					60				
Thr	Val	Arg	Asn	Lys	Leu	Ser	Asp	Pro	Thr	Met	Arg	Arg	Ser	Thr	Thr
65					70					75					80

Ile His Trp His Gly Leu Leu Gln His Arg Thr Ala Glu Glu Asp Gly
 85 90 95
 Pro Ala Phe Val Thr Gln Cys Pro Ile Pro Pro Gln Glu Ser Tyr Thr
 100 105 110
 Tyr Thr Met Pro Leu Gly Glu Gln Thr Gly Thr Tyr Trp Tyr His Ser
 115 120 125
 His Leu Ser Ser Gln Tyr Val Asp Gly Leu Arg Gly Pro Ile Val Ile
 130 135 140
 Met Asp Pro His Asp Pro Tyr Arg Asn Tyr Tyr Asp Val Asp Asp Glu
 145 150 155 160
 Arg Thr Val Phe Thr Leu Ala Asp Trp Tyr His Thr Pro Ser Glu Ala
 165 170 175
 Ile Ile Ala Thr His Asp Val Leu Lys Thr Ile Pro Asp Ser Gly Thr
 180 185 190
 Ile Asn Gly Lys Gly Lys Tyr Asp Pro Ala Ser Ala Asn Thr Asn Asn
 195 200 205
 Thr Thr Leu Glu Asn Leu Tyr Thr Leu Lys Val Lys Arg Gly Lys Arg
 210 215 220
 Tyr Arg Leu Arg Ile Ile Asn Ala Ser Ala Ile Ala Ser Phe Arg Phe
 225 230 235 240
 Gly Val Gln Gly His Lys Cys Thr Ile Ile Glu Ala Asp Gly Val Leu
 245 250 255
 Thr Lys Pro Ile Glu Val Asp Ala Phe Asp Ile Leu Ala Gly Gln Arg
 260 265 270
 Tyr Ser Cys Ile Leu Lys Ala Asp Gln Asp Pro Asp Ser Tyr Trp Ile
 275 280 285
 Asn Ala Pro Ile Thr Asn Val Leu Asn Thr Asn Val Gln Ala Leu Leu
 290 295 300
 Val Tyr Glu Asp Asp Lys Arg Pro Thr His Tyr Pro Trp Lys Pro Phe
 305 310 315 320
 Leu Thr Trp Lys Ile Ser Asn Glu Ile Ile Gln Tyr Trp Gln His Lys
 325 330 335
 His Gly Ser His Gly His Lys Gly Lys Gly His His His Lys Val Arg
 340 345 350
 Ala Ile Gly Gly Val Ser Gly Leu Ser Ser Arg Val Lys Ser Arg Ala
 355 360 365
 Ser Asp Leu Ser Lys Lys Ala Val Glu Leu Ala Ala Ala Leu Val Ala
 370 375 380
 Gly Glu Ala Glu Leu Asp Lys Arg Gln Asn Glu Asp Asn Ser Thr Ile
 385 390 395 400
 Val Leu Asp Glu Thr Lys Leu Ile Pro Leu Val Gln Pro Gly Ala Pro
 405 410 415
 Gly Gly Ser Arg Pro Ala Asp Val Val Val Pro Leu Asp Phe Gly Leu
 420 425 430
 Asn Phe Ala Asn Gly Leu Trp Thr Ile Asn Asn Val Ser Tyr Ser Pro

435					440					445						
Pro	Asp	Val	Pro	Thr	Leu	Leu	Lys	Ile	Leu	Thr	Asp	Lys	Asp	Lys	Val	
450					455					460						
Asp	Ala	Ser	Asp	Phe	Thr	Ala	Asp	Glu	His	Thr	Tyr	Ile	Leu	Pro	Lys	
465					470					475					480	
Asn	Gln	Val	Val	Glu	Leu	His	Ile	Lys	Gly	Gln	Ala	Leu	Gly	Ile	Val	
485					490					495						
His	Pro	Leu	His	Leu	His	Gly	His	Ala	Phe	Asp	Val	Val	Gln	Phe	Gly	
500					505					510						
Asp	Asn	Ala	Pro	Asn	Tyr	Val	Asn	Pro	Pro	Arg	Arg	Asp	Val	Val	Gly	
515					520					525						
Val	Thr	Asp	Ala	Gly	Val	Arg	Ile	Gln	Phe	Arg	Thr	Asp	Asn	Pro	Gly	
530					535					540						
Pro	Trp	Phe	Leu	His	Cys	His	Ile	Asp	Trp	His	Leu	Glu	Glu	Gly	Phe	
545					550					555					560	
Ala	Met	Val	Phe	Ala	Glu	Ala	Pro	Glu	Asp	Ile	Lys	Lys	Gly	Ser	Gln	
565					570					575						
Ser	Val	Lys	Pro	Asp	Gly	Gln	Trp	Lys	Lys	Leu	Cys	Glu	Lys	Tyr	Glu	
580					585					590						
Lys	Leu	Pro	Glu	Ala	Leu	Gln										
595																

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ala	Val	Arg	Asn	Tyr	Lys	Phe	Asp	Ile	Lys	Asn	Val	Asn	Val	Ala	Pro
1				5					10					15	
Asp	Gly	Phe	Gln	Arg	Pro	Ile	Val	Ser	Val						
			20				25								

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ser	Gln	Tyr	Val	Asp	Gly	Leu	Arg	Gly	Pro	Leu	Val	Ile	Tyr	Asp	Pro
1				5					10					15	
Asp	Asp	Asp	His												
20															

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 26 amino acids

- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ser	Arg	Tyr	Asx	Val	Asx	Asx	Ala	Ser	Thr	Val	Val	Met	Leu	Glu	Asx
1				5					10					15	
Trp	Tyr	Arg	Thr	Pro	Ala	Xaa	Val	Leu	Glu						
			20						25						

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ser	Leu	Gly	Pro	Thr	Pro	Asn	Tyr	Val	Asn	Pro	Xaa	Ile	Arg	Asp	Val
1				5					10					15	
Val	Arg	Val	Gly	Gly	Thr	Thr	Val	Val							
			20					25							

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Gly	Leu	Ala	Leu	Val	Phe	Ala	Glu	Ala	Pro	Ser	Gln	Ile	Arg	Gln	Gly
1				5					10					15	
Val	Gln	Ser	Val	Gln	Pro	Asp	Asp	Ala							
			20					25							

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ile Arg Tyr Val Gly Gly Pro Ala Val Xaa Arg Ser Val Ile
1 5 10

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ile Leu Ala Asn Pro Ala
1 5

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 8 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Tyr Glu Ala Pro Ser Leu Pro Thr
1 5

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1912 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Rhizoctonia laccase

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 85..1671

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CTAACGCTTG GTGCGGAGCT CGGATCCACT AGTAACGCGC GCCAGTGTGC TGAATTCGC	60
GGCCGCGTCG ACACCTCCTT CAAG ATG CTT TCT AGC ATT ACC CTC CTA CCT	111
Met Leu Ser Ser Ile Thr Leu Leu Pro	
1 5	
TTG CTC GCT GCG GTC TCA ACC CCC GCC TTT GCT GCC GTC CGC AAC TAT	159
Leu Leu Ala Ala Val Ser Thr Pro Ala Phe Ala Ala Val Arg Asn Tyr	
10 15 20 25	
AAG TTC GAC ATC AAG AAC GTC AAT GTC GCT CCC GAT GGC TTT CAG CGC	207
Lys Phe Asp Ile Lys Asn Val Asn Val Ala Pro Asp Gly Phe Gln Arg	
30 35 40	
TCT ATC GTC TCC GTC AAC GGT TTA GTT CCT GGC ACG TTG ATC ACG GCC	255
Ser Ile Val Ser Val Asn Gly Leu Val Pro Gly Thr Leu Ile Thr Ala	
45 50 55	
AAC AAG GGT GAC ACC TTG CGC ATT AAT GTC ACG AAT CAA CTC ACG GAC	303
Asn Lys Gly Asp Thr Leu Arg Ile Asn Val Thr Asn Gln Leu Thr Asp	
60 65 70	
CCT AGT ATG CGT CGT GCC ACA ACG ATT CAT TGG CAT GGA TTG TTC CAA	351
Pro Ser Met Arg Arg Ala Thr Thr Ile His Trp His Gly Leu Phe Gln	
75 80 85	

GCT ACT ACC GCC GAC GAG GAT GGC CCC GCA TTC GTC ACG CAA TGC CCT Ala Thr Thr Ala Asp Glu Asp Gly Pro Ala Phe Val Thr Gln Cys Pro 90 95 100 105	399
ATT GCG CAA AAT TTG TCC TAT ACA TAC GAG ATC CCA TTG CGC GGC CAA Ile Ala Gln Asn Leu Ser Tyr Thr Tyr Glu Ile Pro Leu Arg Gly Gln 110 115 120	447
ACA GGA ACC ATG TGG TAT CAC GCC CAT CTT GCG AGT CAA TAT GTC GAT Thr Gly Thr Met Trp Tyr His Ala His Leu Ala Ser Gln Tyr Val Asp 125 130 135	495
GGA TTG CGA GGC CCT TTG GTC ATC TAT GAT CCA AAC GAC CCA CAC AAG Gly Leu Arg Gly Pro Leu Val Ile Tyr Asp Pro Asn Asp Pro His Lys 140 145 150	543
TCG CGC TAC GAC GTG GAT GAT GCG AGC ACA GTA GTC ATG CTT GAG GAC Ser Arg Tyr Asp Val Asp Ala Ser Thr Val Val Met Leu Glu Asp 155 160 165	591
TGG TAC CAT ACT CCG GCA CCC GTT CTA GAA AAG CAA ATG TTC TCG ACT Trp Tyr His Thr Pro Ala Pro Val Leu Glu Lys Gln Met Phe Ser Thr 170 175 180 185	639
AAT AAC ACC GCT CTG CTC TCT CCT GTT CCG GAC TCG GGT CTT ATC AAT Asn Asn Thr Ala Leu Leu Ser Pro Val Pro Asp Ser Gly Leu Ile Asn 190 195 200	687
GGC AAA GGG CGC TAT GTG GGC GGT CCC GCA GTT CCC CGG TCA GTA ATC Gly Lys Gly Arg Tyr Val Gly Gly Pro Ala Val Pro Arg Ser Val Ile 205 210 215	735
AAC GTA AAA CGT GGG AAA CGA TAT CGC TTG CGC GTA ATC AAC GCT TCT Asn Val Lys Arg Gly Lys Arg Tyr Arg Leu Arg Val Ile Asn Ala Ser 220 225 230	783
GCT ATC GGG TCG TTT ACC TTT TCG ATC GAA GGA CAT AGT CTG ACT GTC Ala Ile Gly Ser Phe Thr Phe Ser Ile Glu Gly His Ser Leu Thr Val 235 240 245	831
ATT GAG GCC GAT GGG ATC CTG CAC CAG CCC TTG GCT GTT GAC AGC TTC Ile Glu Ala Asp Gly Ile Leu His Gln Pro Leu Ala Val Asp Ser Phe 250 255 260 265	879
CAG ATT TAC GCT GGA CAA CGC TAC TCT GTC ATC GTT GAA GCC AAC CAA Gln Ile Tyr Ala Gly Gln Arg Tyr Ser Val Ile Val Glu Ala Asn Gln 270 275 280	927
ACC GCC GCC AAC TAC TGG ATT CGT GCA CCA ATG ACC GTT GCA GGA GCC Thr Ala Ala Asn Tyr Trp Ile Arg Ala Pro Met Thr Val Ala Gly Ala 285 290 295	975
GGA ACC AAT GCA AAC TTG GAC CCC ACC AAT GTC TTT GCC GTA TTG CAC Gly Thr Asn Ala Asn Leu Asp Pro Thr Asn Val Phe Ala Val Leu His 300 305 310	1023
TAC GAG GGA GCG CCC AAC GCC GAA CCC ACG ACG GAA CAA GGC AGT GCT Tyr Glu Gly Ala Pro Asn Ala Glu Pro Thr Thr Glu Gln Gly Ser Ala 315 320 325	1071
ATC GGT ACT GCA CTC GTT GAA GAG AAC CTC CAT GCG CTC ATC AAC CCT Ile Gly Thr Ala Leu Val Glu Glu Asn Leu His Ala Leu Ile Asn Pro 330 335 340 345	1119
GGC GCT CCG GGC GGC TCC GCT CCC GCA GAC GTT TCC CTC AAT CTT GCA Gly Ala Pro Gly Gly Ser Ala Pro Ala Asp Val Ser Leu Asn Leu Ala 350 355 360	1167

ATT GGG CGC AGC ACA GTT GAT GGG ATT CTT AGG TTC ACA TTT AAT AAC Ile Gly Arg Ser Thr Val Asp Gly Ile Leu Arg Phe Thr Phe Asn Asn 365 370 375	1215
ATC AAG TAC GAG GCT CCT TCG TTG CCC ACG CTC TTG AAG ATT TTG GCA Ile Lys Tyr Glu Ala Pro Ser Leu Pro Thr Leu Leu Lys Ile Leu Ala 380 385 390	1263
AAC AAT GCG AGC AAT GAC GCC GAT TTC ACG CCA AAT GAG CAC ACT ATC Asn Asn Ala Ser Asn Asp Ala Asp Phe Thr Pro Asn Glu His Thr Ile 395 400 405	1311
GTA TTG CCA CAC AAT AAA GTT ATC GAG CTC AAT ATC ACC GGA GGT GCA Val Leu Pro His Asn Lys Val Ile Glu Leu Asn Ile Thr Gly Gly Ala 410 415 420 425	1359
GAC CAC CCT ATC CAT CTC CAC GGC CAT GTG TTT GAT ATC GTC AAA TCA Asp His Pro Ile His Leu His Gly His Val Phe Asp Ile Val Lys Ser 430 435 440	1407
CTC GGT GGT ACC CCG AAC TAT GTC AAC CCG CCA CGC AGG GAC GTA GTT Leu Gly Gly Thr Pro Asn Tyr Val Asn Pro Pro Arg Arg Asp Val Val 445 450 455	1455
CGT GTC GGA GGC ACC GGT GTG GTA CTC CGA TTC AAG ACC GAT AAC CCA Arg Val Gly Gly Thr Gly Val Leu Arg Phe Lys Thr Asp Asn Pro 460 465 470	1503
GGC CCA TGG TTT GTT CAC TGC CAC ATT GAC TGG CAC TTG GAG GCT GGG Gly Pro Trp Phe Val His Cys His Ile Asp Trp His Leu Glu Ala Gly 475 480 485	1551
CTC GCA CTT GTC TTT GCC GAG GCC CCC AGC CAG ATT CGC CAG GGT GTC Leu Ala Leu Val Phe Ala Glu Ala Pro Ser Gln Ile Arg Gln Gly Val 490 495 500 505	1599
CAG TCG GTC CAG CCC AAC AAT GCC TGG AAC CAG CTC TGC CCC AAG TAC Gln Ser Val Gln Pro Asn Asn Ala Trp Asn Gln Leu Cys Pro Lys Tyr 510 515 520	1647
GCG GCT CTT CCT CCC GAT TTG CAG T Ala Ala Leu Pro Pro Asp Leu Gln 525	1672

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 529 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Leu Ser Ser Ile Thr Leu Leu Pro Leu Leu Ala Ala Val Ser Thr 1 5 10 15
Pro Ala Phe Ala Ala Val Arg Asn Tyr Lys Phe Asp Ile Lys Asn Val 20 25 30
Asn Val Ala Pro Asp Gly Phe Gln Arg Ser Ile Val Ser Val Asn Gly 35 40 45
Leu Val Pro Gly Thr Leu Ile Thr Ala Asn Lys Gly Asp Thr Leu Arg 50 55 60

Ile Asn Val Thr Asn Gln Leu Thr Asp Pro Ser Met Arg Arg Ala Thr
 65 70 75 80
 Thr Ile His Trp His Gly Leu Phe Gln Ala Thr Thr Ala Asp Glu Asp
 85 90 95
 Gly Pro Ala Phe Val Thr Gln Cys Pro Ile Ala Gln Asn Leu Ser Tyr
 100 105 110
 Thr Tyr Glu Ile Pro Leu Arg Gly Gln Thr Gly Thr Met Trp Tyr His
 115 120 125
 Ala His Leu Ala Ser Gln Tyr Val Asp Gly Leu Arg Gly Pro Leu Val
 130 135 140
 Ile Tyr Asp Pro Asn Asp Pro His Lys Ser Arg Tyr Asp Val Asp Asp
 145 150 155 160
 Ala Ser Thr Val Val Met Leu Glu Asp Trp Tyr His Thr Pro Ala Pro
 165 170 175
 Val Leu Glu Lys Gln Met Phe Ser Thr Asn Asn Thr Ala Leu Leu Ser
 180 185 190
 Pro Val Pro Asp Ser Gly Leu Ile Asn Gly Lys Gly Arg Tyr Val Gly
 195 200 205
 Gly Pro Ala Val Pro Arg Ser Val Ile Asn Val Lys Arg Gly Lys Arg
 210 215 220
 Tyr Arg Leu Arg Val Ile Asn Ala Ser Ala Ile Gly Ser Phe Thr Phe
 225 230 235 240
 Ser Ile Glu Gly His Ser Leu Thr Val Ile Glu Ala Asp Gly Ile Leu
 245 250 255
 His Gln Pro Leu Ala Val Asp Ser Phe Gln Ile Tyr Ala Gly Gln Arg
 260 265 270
 Tyr Ser Val Ile Val Glu Ala Asn Gln Thr Ala Ala Asn Tyr Trp Ile
 275 280 285
 Arg Ala Pro Met Thr Val Ala Gly Ala Gly Thr Asn Ala Asn Leu Asp
 290 295 300
 Pro Thr Asn Val Phe Ala Val Leu His Tyr Glu Gly Ala Pro Asn Ala
 305 310 315 320
 Glu Pro Thr Thr Glu Gln Gly Ser Ala Ile Gly Thr Ala Leu Val Glu
 325 330 335
 Glu Asn Leu His Ala Leu Ile Asn Pro Gly Ala Pro Gly Gly Ser Ala
 340 345 350
 Pro Ala Asp Val Ser Leu Asn Leu Ala Ile Gly Arg Ser Thr Val Asp
 355 360 365
 Gly Ile Leu Arg Phe Thr Phe Asn Asn Ile Lys Tyr Glu Ala Pro Ser
 370 375 380
 Leu Pro Thr Leu Leu Lys Ile Leu Ala Asn Asn Ala Ser Asn Asp Ala
 385 390 395 400
 Asp Phe Thr Pro Asn Glu His Thr Ile Val Leu Pro His Asn Lys Val
 405 410 415
 Ile Glu Leu Asn Ile Thr Gly Gly Ala Asp His Pro Ile His Leu His

420					425					430					
Gly	His	Val	Phe	Asp	Ile	Val	Lys	Ser	Leu	Gly	Gly	Thr	Pro	Asn	Tyr
		435					440					445			
Val	Asn	Pro	Pro	Arg	Arg	Asp	Val	Val	Arg	Val	Gly	Gly	Thr	Gly	Val
	450					455					460				
Val	Leu	Arg	Phe	Lys	Thr	Asp	Asn	Pro	Gly	Pro	Trp	Phe	Val	His	Cys
465					470					475					480
His	Ile	Asp	Trp	His	Leu	Glu	Ala	Gly	Leu	Ala	Leu	Val	Phe	Ala	Glu
				485					490					495	
Ala	Pro	Ser	Gln	Ile	Arg	Gln	Gly	Val	Gln	Ser	Val	Gln	Pro	Asn	Asn
			500					505					510		
Ala	Trp	Asn	Gln	Leu	Cys	Pro	Lys	Tyr	Ala	Ala	Leu	Pro	Pro	Asp	Leu
		515					520					525			
Gln															

What we claim is:

1. A nucleic acid fragment containing a nucleic acid sequence encoding a *Rhizoctonia* laccase which functions optimally at
5 pH between about 6.0 and 8.5.

2. The fragment of Claim 1 which comprises a sequence encoding a *Rhizoctonia solani* laccase.

10 3. The fragment of Claim 1 which comprises a nucleic acid sequence encoding the amino acid sequence depicted in SEQ ID NO. 2.

4. The fragment of Claim 1 which comprises a nucleic acid
15 sequence encoding the amino acid sequence depicted in SEQ ID NO. 4.

5. The fragment of Claim 1, which comprises a nucleic acid sequence encoding a protein containing one or more of the
20 amino acid sequences depicted in SEQ. ID NOS. 5, 6, 7, 8, 9, 10, 11, or 12.

6. The fragment of Claim 1 which comprises a nucleic acid sequence encoding the amino acid sequence depicted in SEQ ID
25 NO. 14.

7. The fragment of Claim 1, which comprises the nucleic acid sequence depicted in SEQ ID NO. 1.

30 8. The fragment of Claim 1, which comprises the nucleic acid sequence depicted in SEQ. ID. NO. 3.

9. The fragment of Claim 1, which comprises the nucleic acid sequence depicted in SEQ. ID. NO. 13.
10. The fragment of Claim 1, which comprises the nucleic acid sequence contained in NRRL B-21141.
11. The fragment of Claim 1, which comprises the nucleic acid sequence contained in NRRL B-21142.
- 10 12. The fragment of Claim 1, which comprises the nucleic acid sequence encoding the laccase produced by RS 22.
13. The fragment of Claim 1, which comprises the nucleic acid sequence contained in NRRL B-21156.
- 15 14. A substantially pure *Rhizoctonia* laccase enzyme which functions optimally at a pH between about 6.0-8.5.
- 15 15. The enzyme of Claim 14 which is a *Rhizoctonia solani* laccase.
- 20 16. The enzyme of Claim 14 which comprises the sequence depicted in SEQ ID NO. 2, or a sequence with at least 80% homology thereto.
- 25 17. The enzyme of Claim 14 which comprises the sequence depicted in SEQ ID NO 4, or a sequence with at least 80% homology thereto.
- 30 18. The enzyme of Claim 14 which comprises one or more of the peptide sequences depicted in SEQ ID NOS.5, 6, 7,

8, 9, 10, 11 or 12, or a sequence with at least 80% homology to one or more of these peptides.

19. The enzyme of Claim 14 which comprises the sequence
5 depicted in SEQ ID NO 14, or a sequence with at least 80% homology thereto.

20. A recombinant vector comprising a nucleic acid fragment containing a nucleic acid sequence encoding a *Rhizoctonia*
10 laccase which functions optimally at pH between about 6.0-8.5.

21. The vector of Claim 20 in which the fragment is operably linked to a promoter sequence.

15

22. The vector of Claim 21 in which the promoter is a fungal or yeast promoter.

23. The vector of Claim 22 in which the promoter is the
20 TAKA amylase promoter of *Aspergillus oryzae*.

24. The vector of Claim 22 in which the promoter is the glucoamylase (gluA) promoter of *Aspergillus niger* or *Aspergillus awamsii*.

25

25. The vector of Claim 21 which also comprises a selectable marker.

26. The vector of Claim 25 in which the selectable marker
30 is the amdS marker of *Aspergillus nidulans* or *Aspergillus oryzae*.

27. The vector of Claim 25 in which the selectable marker is the pyrG marker of *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus awamorii*, or *Aspergillus oryzae*.
- 5 28. The vector of Claim 21 which comprises both the TAKA amylase promoter of *Aspergillus oryzae* and the amdS or pyrG marker of *Aspergillus nidulans* or *Aspergillus oryzae*.
- 10 29. A host cell comprising a heterologous nucleic acid fragment containing a nucleic acid sequence encoding a *Rhizoctonia* laccase which functions optimally at pH between about 6.0-8.5.
- 15 30. The host cell of Claim 28 which is a fungal cell.
31. The host cell of Claim 30 which is an *Aspergillus* cell.
- 20 32. The host cell of Claim 29 in which the fragment is integrated into the host cell genome.
33. The host cell of Claim 29 in which the fragment is contained on a vector.
- 25 34. The host cell of Claim 29 which comprises a fragment containing a sequence encoding the amino acid sequence depicted in SEQ ID NO. 2.
- 30 35. The host cell of Claim 29 which comprises a fragment containing a sequence encoding the amino acid sequence depicted in SEQ ID NO: 4.

36. The host cell of Claim 29 which comprises a fragment containing a sequence encoding the amino acid sequence depicted in SEQ ID NO: 14.
- 5 37. The host cell of Claim 29 which comprises a fragment containing a sequence encoding one or more of the amino acid sequences depicted in SEQ ID NOS.: 5, 6, 7, 8, 9, 10, 11, or 12.
- 10 38. A method for obtaining a laccase enzyme which functions optimally at a pH between about 6.0-8.5 which comprises culturing a host cell comprising a nucleic acid fragment containing a nucleic acid sequence encoding a *Rhizoctonia* laccase enzyme which functions optimally at a pH between
15 about 6.0-8.5, under conditions conducive to expression of the enzyme, and recovering the enzyme from the culture.
39. A method for polymerizing a lignin or lignosulfate substrate in solution which comprises contacting the
20 substrate with a *Rhizoctonia* laccase which functions optimally at a pH between about 6.0-8.5.
40. A method for in situ depolymerization in Kraft pulp which comprises contacting the pulp with a *Rhizoctonia*
25 laccase which functions optimally at a pH between about 6.0-8.5.
41. A method for oxidizing dyes which comprises contacting the dye with a *Rhizoctonia* laccase which functions optimally
30 at a pH between about 6.0-8.5.

42. A method of polymerizing a phenolic compounds which comprises contacting the phenolic compound with a *Rhizoctonia* laccase which functions optimally at a pH between about 6.0-8.5.

5

1 AGCGTCACACCAGACATCGGATGAAACGGAAAGTGATATGCGCCATTGTGACGCTGCGGC 60
61 AACCACTGTTTCATCTCGCGAGCTAACATGGCGACGTATAAGAAACGCGAGAAATGGGC 120
121 AGATTTCGATATCCCCCTCTCGTCTCGGTTTGGTCTCGGCTTGCCCTCTAATGGCGGCAC 180
M A R T
181 CACTTTCCTTGTCTCGGTTTCGCTCTTTTGTTCCTCGCTGTTCTTGGCGGCACCGTCGAGTA 240
4 T F L V S V S L F V S A V L A R T V E Y 24
241 CGGCTTGAAGATTAGTGATGGGAGATAGCTCTGACGGTGTTAAGCGTAATGCGACTTT 300
24 G L K I S D G E I A P D G V K R N A T L 44
2301 GGgtacgcactccttgtaatccaacaattcaaggtttctgatgcttggtcagTAAATGGA 360
V N G 47
361 GGGTATCCCGGTCCACTCATTTTGGCCAAACAAGGGGATACTCTCAAAGTCAAGGTCCAA 420
47 G Y P G P L I F A N K G D T L K V K V Q 67
421 AACAAAGCTCACGAATCCTGAGATGTATCGCACCACTTCCATCgtatgttcgattcgcatac 480
67 N K L T N P E M Y R T S I 81
481 tactaatacatccgctcgctaaatatctttagCATTTGGCACGGTCTCTTACAACATAGAA 540
H W H G L L Q H R 90
81

FIG. 1A

541 ACGCCGACGACGAGCGGTCCTTCGTTGTCACCTCAGgtaggattcttggagggttggcctga 600
 90 N A D D D G P S F V T Q 102

 601 actctctgttaaccgacaaccgcatgtcaccagTGCCCGATGTTCACGCGAGTCGTAT 660
 102 C P I V P P R E S Y 111

 661 ACTTACACCATACCTCTGGACGATCAAAACCGGAACCTATTGGTACCATAGCCACTTGAGT 720
 111 T Y T I P L D D Q T G T Y W Y H S H L S 131

 721 TCGCAATACGTTGATGGTCTTCGAGGCCCGCTGGTAATCTgtgagtatcttgacttgtct 780
 131 S Q Y V D G L R G P L V I 144

 781 actgaaggcaacgagactaaaacaagcgctcgattcacagATGgttcgtctccccctttatt 840
 144 Y 145

 841 tagctctggatcttcatttctcacgtaatacatgatagATCCCAAGGATCCTCACAGCG 900
 144 D P K D P H R R 152

 901 TTTGTATGATGTTGACGATGAGAAGACCGTCCTGATCATCGGTGACTGGTATCATGAATC 960
 152 L Y D V D D E K T V L I I G D W Y H E S 172

 961 GTCCAAGGCAATCCTTGCTTCTGGTAACATTACCCGACagtaagtgatcacatgccgggtcc 1020
 172 S K A I L A S G N I T R Q 185

FIG. 1B

1021 cagaaaaattctctaaattcattttaaattacagcggacccggctctctgcccaccatcaacgg 1030
 185 R P V S A T I N G 194
 1081 CAAAGGTCGATTGTGACCCCTGACAACACTCCTGCCAACCCAGATACTCTGTACACCCCTCAA 1140
 194 K G R F D P D N T P A N P D T L Y T L K 214
 1141 GGTCAGCGAGGGAAGCGCTATCGTCTGCGTGTCAATCAATAGCTCGGAGATCGCTTCGTT 1200
 214 V K R G K R Y R L R V I N S S E I A S F 234
 1201 CCGATTCAAGTGTGGAAGGTCACAAGGTGACTGTGATTGCTGCCGATGGCGTCTCTACCAA 1260
 234 R F S V E G H K V T V I A A D G V S T K 254
 1261 ACCGTATCAGGTCGATGCGTTTGATATTCTAGCAGGACAGCGCATAGATTGCGTCgtaag 1320
 254 P Y Q V D A F D I L A G Q R I D C V 272
 1321 tgtcgtccgaaccacacatctgagctcaagtgtgatacatgcgcgcttatagGTGGAGGC 1380
 272 V E A 275
 1381 GAACCAAGAACCCGACACATACTGGATCAACGCACCCGCTGACCAACGTGCCCAACAAGAC 1440
 275 N Q E P D T Y W I N A P L T N V P N K T 295
 1441 CGCTCAGGCTCTCCCTCGTTTATGAGGAGGATCGTCGGCCGTACCACCCCTCCAAAGGGCCC 1500
 295 A Q A L L V Y E E D R R P Y H P P K G P 315
 1501 GTATCGCAAGTGGAGCGTCTCTGAGGCGGATCATCAAGTACTGGAATCACAAGCACAAAGCA 1560
 315 Y R K W S V S E A I I K Y W N H K H K H 335

FIG. 1C

1561 CGGACGTGGTTGCTGCTGACATGGAGGCTCTCAAGGCTCGGATGATCGAGGGTAGCCA 1620
 335 G R G L L S G H G G L K A R M I E G S H 340
 1621 TCATCTGCATTGCGCGAGCGTCGTTAAGCGCCAGAAATGAGACCACCACCTGTTGTAATGGA 1680
 340 H L H S R S V V K R Q N E T T T V V M D 350
 1681 CGAGAGCAAGCTCGTTGtaagaccataatttaaaagttgggttgcgaatacttatt 1740
 350 E S K L V
 1741 tcaacttttcttagCCACTGGAATACCCCGCGCTGCATGCGGGTCTAAACCTGCTGACC 1800
 350 P L E Y P G A A C G S K P A D 365
 1801 TCGTCTTGGATCTCATTGTTGTTGtatgtagccaaatcgcccatatacaggatactg 1860
 365 L V L D L T F G L 374
 4 1861 aatattgttgtgcgtgtagAACTTTGCTACCGGGCACCTGGATGATCAACGGTATCCCAT 1920
 374 N F A T G H W M I N G I P 387
 1921 ACGAGTCTCCCAAAATCCCCACATTGCTCAAGATCCCTCACTGATGAGGACGGGGTTACCG 1980
 387 Y E S P K I P T L L K I L T D E D G V T 407
 1981 AGTCTGACTTgtatgttcccttttccggtatcttcgtatgcgtgcactgactcgtgctggt 2040
 407 E S D F 411
 2041 gggaaatttagCACCAGGAGGAGCACACAGTCATACTCCCGAAGAACAAATGCATCGAAT 2100
 411 T K E E H T V I L P K N K C I E 427

FIG. 1D

2101 TCAACATCAAGGGAACCTCGGTATTCCCATTAACGCACCCCGTACATCTTACGGTgtaa 2160
 427 F N I K G N S G I P I T H P V H L H G 446
 2161 gtgcataatcggtgttacgataactaaggctcatcaacttttttagCACACTTGGGATGT 2220
 446 H T W D V 451
 2221 CGTACAAATTGGCAACAACCCACCCAAATTATGTCAATCCTCCCGTAGGACGTGGTTGG 2280
 451 V Q F G N N P P N Y V N P P R R D V V G 471
 2281 CTCTACAGATCGGGTGTGAGGATTCAGTTCAAGACCGACAATCCAGACCGTGGTTTCCT 2340
 471 S T D A G V R I Q F K T D N P G P W F L 491
 2341 GCACTGgtgcgtcggcccccatcgccccgttatgggttttctaatatcgccccattctattt 2400
 491 H C 493
 2401 tagCCATATTGACTGGCATCTTGAGGAGGGTTTCGCAAGtgagtactgagacctaaagtgc 2460
 493 H I D W H L E E G F A 504
 2461 tactcggctcattactgattaccgcatgtatgcgtctagTGGTGTGTGCTGAAGCGCCCG 2520
 504 M V F A E A P 511
 2521 AAGCCGTCAAGGAGGTCCAAAGAGCGTGGCCGTGGACTCTCAGTGGGAAGGGCTGTGTG 2580
 511 E A V K G G P K S V A V D S Q W E G L C 531
 2581 GCAAGTACGACAACCTGGCTAAATCAATCCGGGCCAGCTGTAGGCGTATCGCAGCCACA 2640
 531 G K Y D N W L K S N P G Q L * 545

FIG. 1E

2641 TTGGTGATGATTGAAAGTTGCATCTTGTTCCTATAACCGGCTCTTATATACGGGTGTC TC 2700

2701 CCAGTAAAGTCGTAGCCCCAATTTCAGCCGAGACAGATATTAGTGGACTCTTACTCTTGT 2760

2761 GTCCCATTGACGCACATCGTTGCATCAAAACCTGCTTTTATCGTCCCCTCTTGTAAATTG 2820

2821 TGTTCCTGTAATGTATCG 2838

FIG. 1F

```

1  AAGCTTCGGCATGGATTGCCATTTTGTATTGT      180
181 AAACAAGTTACGAGAAAACAATAGATCAGTTTTTTGCCGAATCGGATGGCTTGAAACGGA      240
241 AGTACCGATGGCCGATCCGAGTCGAATGAATTAACGCATCTGAAACGGGACCCCTGAGTCG      300
301 AGGCACCCGCCGCTTGGCCGTATAAGTCACTTGTCGCCCAACTAGCACATTTTTCATTCC      360
361 CCCTTTTCTTCTTCCTCGTCTTCTTCTCTATGGCTCGGTCGACTACTTCACTCTTTG      420
      1      M A R S T T S L F
421 CACTGTCTCTGGCCGCACCGCCCTTGGCTCGAGTCGTTGACTATGGGTTTGTATGTGGCTA      480
      10 A L S L A A P A L A R V V D Y G F D V A
481 ATGGGGCAGTTGCTCCGGATGGTGTAACAAGGAACGCGGTTCTCGgtgagctgtaa      540
      30 N G A V A P D G V T R N A V L
541 gatggtgtatatgctggtgtgcctaacgggaatgtcagTCAATGGTCGCTTCCCCTGGTCCA      600
      45      V N G R F P G P
601 TTGATCACCGCCAACAAGGGGGATACACTTAAATCACCGTGCAGAAATAAACTCTCCGAT      660
      53 L I T A N K G D T L K I T V R N K L S D

```

FIG. 2A

661 CCAACTATGCGAAGGAGCACGACCATCGttagtacttccccctcatctgtcttgaaacttt 720
 73 P T M R R S T T I 82

 721 ctcatcttttttgaagCACTGGCAGGTCTGTCTCCAACACAGGACGGCAGAGAAGATGG 780
 82 H W H G L L Q H R T A E D G 97

 781 CCCGGCCTTTGTAAACCCAGgtatgccttatcctatcgctgtctgtcccccggtccttcc 840
 97 P A F V T Q 103

 841 ctgactcgggcgattcttagTGCCCCGATTCTCCGCAAGAAATCGTACACCTATACGATGCC 900
 103 C P I P P Q E S Y T Y T M P 117

 901 GCTCGGCGAACAGACCGGCACGTATTGGTACCACAGCCACTTGAGCTCCAGTATGTGGA 960
 117 L G E Q T G T Y W Y H S H L S S Q Y V D 137

 961 CGGGTTGCGTGCGGCCCATCGTTATTgttaagtcttcatttaaccttattcttggtatgg 1020
 137 G L R G P I V I 145

 1021 ctgattgtgacgtcgtggtagATGgttcggtggcttccacaagaagtcagcagcccttga 1080
 145 Y 145

 1081 agctaaactttattccagACCCCCACGACCCGTACAGAAACTACTATGATGTCGACGACGA 1140
 145 D P H D P Y R N Y Y D V D D E 160

 1141 GCGTACGGTCTTTACTTTAGCAGACTGGTACCACACGCCGTGGAGGCTATCATGTCAC 1200
 160 R T V F T L A D W Y H T P S E A I I A T 180

FIG. 2B


```

1201 CCACGATGTCTTGAAACGtacgcgtaataccttctagctttcttcccttgggtcacttt 1260
180   H D V L K T 185

1261 ctatcagGATCCCCGACTCGGGTACGATCAACGGCAAGCAATAACGATCCTGCTTCGG 1320
185   I P D S G T I N G K G K Y D P A S 202

1321 CTAACACCAACAACACGACACTCGAGAACCTCTACACTCTCAAAGTCAAACGGCGCAAGC 1380
202 A N T N N T T L E N L Y T L K V K R G K 222

1381 GGTATCGCCTGAGGATTATCAACGCCCTCGGCCATCGTTCGTTCCGGTTCGGCGTGCAGG 1440
222 R Y R L R I I N A S A I A S F R F G V Q 242

1441 GCCACAAGTGCACGATCATCGAGGCTGATGGCGTCTCCTCACCAAACCGATCGAGTCGATG 1500
242 G H K C T I I E A D G V L T K P I E V D 262

1501 CGTTTGATATTCTAGCAGGCCAGAGGTATAGCTGCATCgtaagtctaccctatgacctgtt 1560
262 A F D I L A G Q R Y S C I 275

1561 gtggagataagaacctgactgaatgtatgcgctccaatagTTGAAGGCCGACCAAGATCC 1620
275   L K A D Q D P 282

1621 TGATTCCCTACTGGATAAATGCGCCAATCACAAACGTTCTCAACACCAACGTCAGGCATT 1680
282   D S Y W I N A P I T N V L N T N V Q A L 302

1681 GCTAGTGTATGAAGATGACAAGCGTCCCTACTACTACCCCTGGAAGCCGTTTGTGACATG 1740
302   L V Y E D D K R P T H Y P W K P F L T W 322

```

FIG. 2C

1741 GAAGATATCAAAATGAAATCATTCAGTACTGGCAGCACAAAGCACGGGTGCGACGGTCACAA 1800
322 K I S N E I I Q Y W Q H K H G S H G H K 342
1801 GGGAAAGGGGCATCATATAAGTCCGGCCATTGGAGGTGTATCCGGGTGAGCTCCAG 1860
342 G K G H H K V R A I G G V S G L S S R 362
1861 GGTTAAGAGCCGGCGAGTGACCTATCGAAGAAGGCTGTGAGTTGGCTGCTGCACTCGT 1920
349 V K S R A S D L S K K A V E L A A A L V 349
1921 TCGGGGTGAGGCCGAGTTGGACAAGAGGCAGAAATGAGGATAATTCGACTATTGTATTGGA 1980
349 A G E A E L D K R Q N E D N S T I V L D 361
1981 TGAGACCAAGCTTATgtgaagtccttaatttttttcgggtgtcacggaagctaaccgcg 2040
361 E T K L I 361
2041 taatagCCGTTGGTTCAACCTGGTGCACCGGGCGGCTCCAGACCAGCTGACGTGCTGGTC 2100
361 P L V Q P G A P G G S R P A D V V 379
2101 CCTCTGGACTTTGGCCCTCgtatgtggcttcttgttatttcgtccggaatgcaaaactgattt 2160
379 P L D F G L 385
2161 ggggtgggctatagAACTTTGCCAACGGACTGTGGACGATAAAACAATGTCTCCTACTCCCC 2220
385 N F A N G L W T I N N V S Y S P 401
2221 TCCGGATGTCCCTACTCTCAAGATCTTGACCGACAAAGACAAAGTCGACGCTTCTGA 2280
401 P D V P T L L K I L T D K D K V D A S D 421

FIG. 2D

2281 CTTgtaggttcctctcttcttcttcttcaactagctactgacattaagtgaacgtcagCACG 2340
 421 F T
 2341 GCCGATGAACACACGTATATTTCTCCAAAGAACCAAGTGTGCGAGTTGCACATCAAGGGA 2400
 423 A D E H T Y I L P K N Q V V E L H I K G 453
 2401 CAGGCTTTGGGAATCGTACACCCCTTCATCTGCATGGCGgtacgtcttctcacactggtt 2460
 453 Q A L G I V H P L H L H G 466
 2461 ccagctccctattctctaacacacactcctcgcatagCATGCGTTCGACGTCGTCCAATTCGG 2520
 466 H A F D V V Q F G 475
 2521 CGACAAACGCTCCAAACTACGTGAACCCCTCCGCGTAGGGATGTAGTGGCGTAACGTGATGC 2580
 475 D N A P N Y V N P P R R D V V G V T D A 495
 2581 TGGAGTCCGTATCCAGTTCAGAACCGATAACCCGGCCCTTGCTTCCCTCCATTGgtatgc 2640
 495 G V R I Q F R T D N P G P W F L H C 513
 2641 tcttcatctcccaccgcttggttcttacttatgggtttacccttgcgatttagCCACATTGA 2700
 513 H I D 516
 2701 TTGGCACTTGGGAAGAAGGATTTGTAGtaagttattatcctattccgaagcatcgggga 2760
 516 W H L E E G F A 524
 2761 gatgctaaccaagggtgtgtttaaagTGGTATTCGCCGAAGCGCCTGAAGATATCAAGAA 2820
 524 M V F A E A P E D I K K 536

FIG. 2E

2821 AGGCTCTCAGAGTGTCAAGCCTGACGGACAATGGAAGAACTATGCGAGAAGTATGAGAA 2880
536 G S Q S V K P D G Q W K K L C E K Y E K 556
2881 GTTGCCCTGAAGCACTGCAGTGAAGTTGCAGTTGTTCCCATTCGGGAAC TGGCTCACTAT 2940
556 L P E A L Q * 562
2941 TCCTTTTGCATAAATTCGGACTTTTATTTTGGGACATTATTGGACTATGCATTGTGTTGTC 3000

3001 ACACCGCGGAAC TAAGCCGAATTC

FIG. 2F

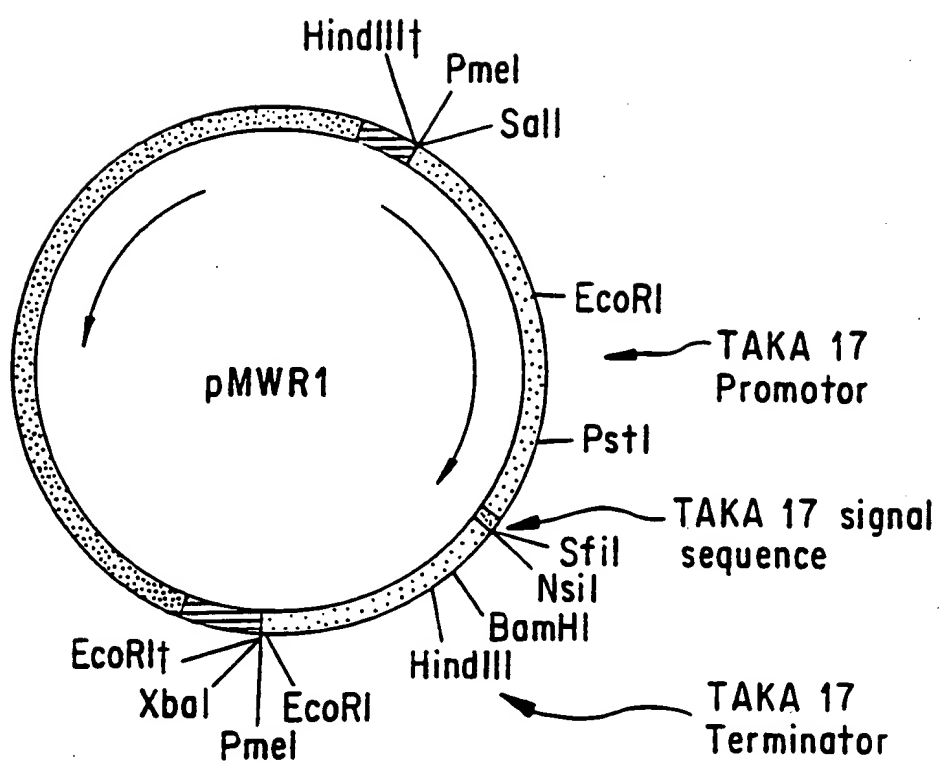


FIG. 3

5' ATG CTT TCT AGC ATT ACC CTA CCT TTG CTC GCT GCG GTC TCA ACC CCC GCC
 87 96 105 114 123 132
 --- M L S S I T L L P L L A A V S T P A

 TTT GCT GCC GTC CGC AAC TAT AAG TTC GAC ATC AAG AAC GTC AAT GTC GCT CCC
 141 150 159 168 177 186
 --- F A A V R N Y K F D I K N V N V A P

 GAT GGC TTT CAG CGC TCT ATC GTC TCC GTC AAC GGT TTA GTT CCT GGC ACG TTG
 195 204 213 222 231 240
 --- D G F Q R S I V S V N G L V P G T L

 ATC ACG GCC AAC AAG GGT GAC ACC TTG CGC ATT AAT GTC ACG AAT CAA CTC ACG
 249 258 267 276 285 294
 --- I T A N K G D T L R I N V T N Q L T

 GAC CCT AGT ATG CGT CGT GCC ACA ACG ATT CAT TGG CAT GGA TTG TTC CAA GCT
 303 312 321 330 339 348
 --- D P S M R R A T T I H W H G L F Q A

FIG. 4A

357	366	375	384	393	402
ACT ACC GCC GAC GAG GAT GGC CCC GCA TTC GTC ACG CAA TGC CCT ATT GCG CAA					
---	---	---	---	---	---
T T A D E D G P A F V T Q C P I A Q					
411	420	429	438	447	456
AAT TTG TCC TAT ACA TAC GAG ATC CCA TTG CGC GGC CAA ACA GGA ACC ATG TGG					
---	---	---	---	---	---
N L S Y T Y E I P L R G Q T G T M W					
465	474	483	492	501	510
TAT CAC GCC CAT CTT GCG AGT CAA TAT GTC GAT GGA TTG CGA GGC CCT TTG GTC					
---	---	---	---	---	---
Y H A H L A S Q Y V D G L R G P L V					
519	528	537	546	555	564
ATC TAT GAT CCA AAC GAC CCA CAC AAG TCG CGC TAC GAC GTG GAT GAT GCG AGC					
---	---	---	---	---	---
I Y D P N D P H K S R Y D V D D A S					
573	582	591	600	609	618
ACA GTA GTC ATG CTT GAG GAC TGG TAC CAT ACT CCG GCA CCC GTT CTA GAA AAG					
---	---	---	---	---	---
T V V M L E D W Y H T P A P V L E K					

FIG. 4B

CAA	ATG	TTC	TCG	ACT	AAT	AAC	ACC	GCT	645	636	645	654	663	672
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Q	M	F	S	T	N	N	T	A	L	L	S	P	P	G
CTT	ATC	AAT	GGC	AAA	GGG	CGC	TAT	GTG	699	690	708	717	726	726
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
L	I	N	G	K	G	R	Y	V	G	G	P	A	P	V
ATC	AAC	GTA	AAA	CGT	GGG	AAA	CGA	TAT	753	744	762	771	780	780
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
I	N	V	K	R	G	K	R	Y	R	L	R	V	I	A
ATC	GGG	TCG	TTT	ACC	TTT	TCG	ATC	GAA	807	798	816	825	834	834
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
I	G	S	F	T	F	S	I	E	G	H	S	L	T	A
GAT	GGG	ATC	CTG	CAC	CAG	CCC	TTG	GCT	861	852	870	879	888	888
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
D	G	I	L	H	Q	P	L	A	V	D	S	F	Q	G
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

FIG. 4C

897	906	915	924	933	942
CAA CGC TAC	TCT GTC ATC	GTT GAA GCC AAC CAA ACC GCC AAC	TAC TGG ATT		
Q R Y S V I V E A N Q T A A N Y W I					
951	960	969	978	987	996
CGT GCA CCA ATG ACC GTT GCA GGA GCC GGA ACC AAT GCA AAC TTG GAC CCC ACC					
R A P M T V A G A G T N A N L D P T					
1005	1014	1023	1032	1041	1050
AAT GTC TTT GCC GTA TTG CAC TAC GAG GGA GCG CCC AAC GCC GAA CCC ACG ACG					
N V F A V L H Y E G A P N A E P T T					
1059	1068	1077	1086	1095	1104
GAA CAA GGC AGT GCT ATC GGT ACT GCA CTC GTT GAA GAG AAC CTC CAT GCG CTC					
E Q G S A I G T A L V E E N L H A L					
1113	1122	1131	1140	1149	1158
ATC AAC CCT GGC GCT CCG GGC GGC TCC GCT CCC GCA GAC GTT TCC CTC AAT CTT					
I N P G A P G G S A P A D V S L N L					

FIG. 4D

1167	1176	1185	1194	1203	1212
GCA ATT GGG CGC AGC ACA GTT GAT GGG ATT CTT AGG TTC ACA TTT AAT AAC ATC					
---	---	---	---	---	---
A I G R S T V D G I L R F T F N N I					
1221	1230	1239	1248	1257	1265
AAG TAC GAG GCT CCT TCG TTG CCC ACG CTC TTG AAG ATT TTG GCA AAC AAT GCG					
---	---	---	---	---	---
K Y E A P S L P T L L K I L A N N A					
1275	1284	1293	1302	1311	1320
AGC AAT GAC GCC GAT TTC ACG CCA AAT GAG CAC ACT ATC GTA TTG CCA CAC AAT					
---	---	---	---	---	---
S N D A D F T P N E H T I V L P H N					
1329	1338	1347	1356	1365	1374
AAA GTT ATC GAG CTC AAT ATC ACC GGA GGT GCA GAC CAC CCT ATC CAT CTC CAC					
---	---	---	---	---	---
K V I E L N I T G G A D H P I H L H					
1383	1392	1401	1410	1419	1428
GGC CAT GTG TTT GAT ATC GTC AAA TCA CTC GGT GGT ACC CCG AAC TAT GTC AAC					
---	---	---	---	---	---
G H V F D I V K S L G G T P N Y V N					

FIG. 4E

1437	1446	1455	1464	1473	1482
CCG CCA CGC AGG GAC GTA GTT CGT GTC GGA GGC ACC GGT GTG GTA CTC CGA TTC					
---	---	---	---	---	---
P P R R D V V R V G G T G V V L R F					
1491	1500	1509	1518	1527	1536
AAG ACC GAT AAC CCA GGC CCA TGG TTT GTT CAC TGC CAC ATT GAC TGG CAC TTG					
---	---	---	---	---	---
K T D N P G P W F V H C H I D W H L					
1545	1554	1563	1572	1581	1590
GAG GCT GGG CTC GCA CTT GTC TTT GCC GAG GCC CCC AGC CAG ATT CGC CAG GGT					
---	---	---	---	---	---
E A G L A L V F A E A P S Q I R Q G					
1599	1608	1617	1626	1635	1644
GTC CAG TCG GTC CAG CCC AAC AAT GCC TGG AAC CAG CTC TGC CCC AAG TAC GCG					
---	---	---	---	---	---
V Q S V Q P N N A W N Q L C P K Y A					
1653	1662				
GCT CTT CCT CCC GAT TTG CAG T 3'					
---	---				
A L P P D L Q					

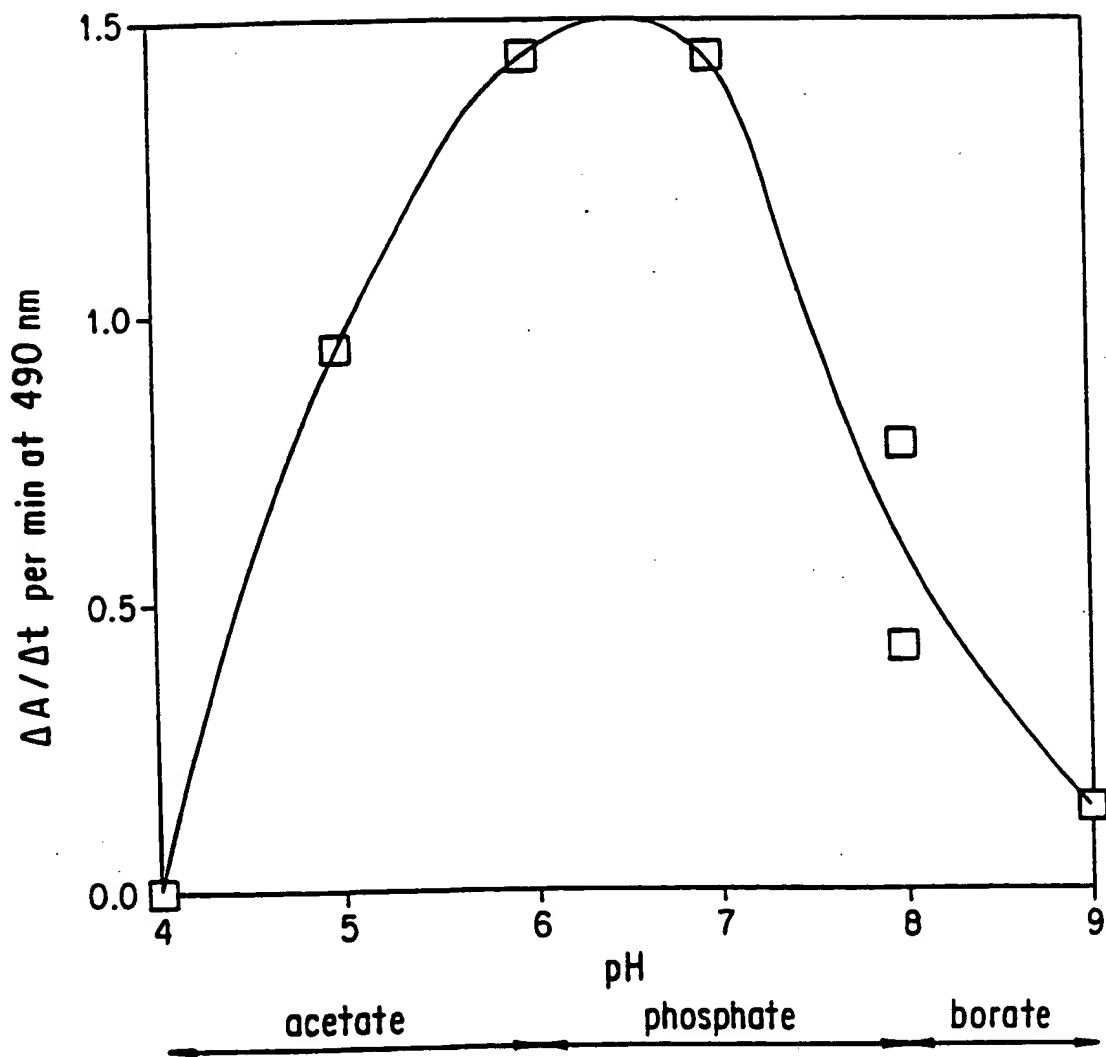


FIG. 5

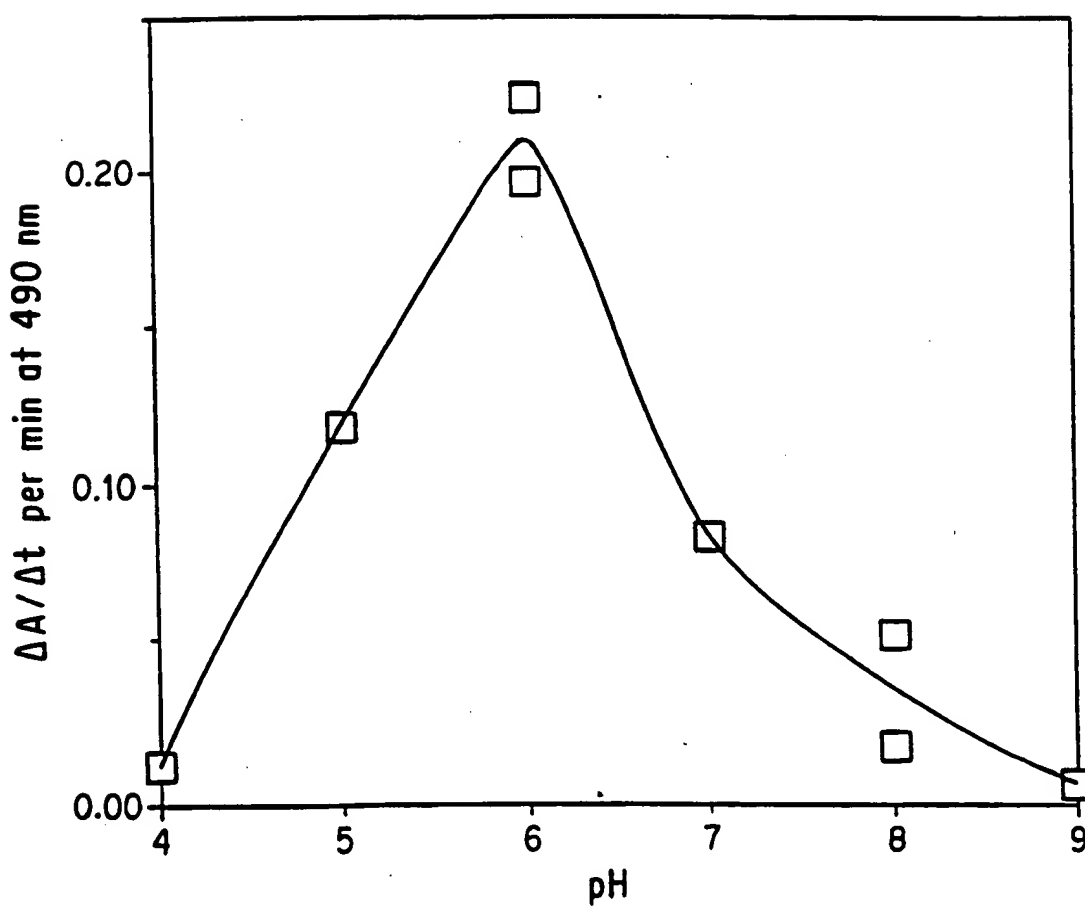


FIG. 6

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/53 C12N9/02 C12N15/80 D21C5/00 A61K7/06
 C12P7/22 C12N1/19 C09B69/10 //(C12N1/19, C12R1:66)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N D21C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 90, no. 19, 7 May 1979, Columbus, Ohio, US; abstract no. 147536w, BOLLAG J.M. ET AL. 'Characterization of an enzyme from Rhizoctonia praticola which polymerizes phenolic compounds.' page 213 ; see abstract	14,43
Y	& CAN. JOURNAL MICROBIOL., vol.25, no.2, 1979 pages 229 - 223 --- -/--	1,20-24, 39-41

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

A document member of the same patent family

Date of the actual completion of the international search

24 January 1995

Date of mailing of the international search report

23. 02. 95

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+31-70) 340-3016

Authorized officer

DeLanghe, L

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 100, no. 19, 7 May 1984, Columbus, Ohio, US; abstract no. 152972q, LEONOWICZ A. ET AL. 'The effect of pH on the transformation of syringic and vanillic acids by the laccases of Rhizoctonia praticola and Trametes versicolor.' page 230 ; see abstract	14,43
Y	& ARCH.MICROBIOL., vol.137, no.2, 1984 pages 89 - 96	1,20-24, 39-41
Y	WO,A,92 01046 (VALTION TEKNILLINEN TUTKIMUSKESKUS) 23 January 1992 see claims	1,20,21
Y	WO,A,92 16633 (NOVO NORDISK) 1 October 1992 see page 3; claims	21-24
Y	DE,A,30 37 992 (GESELLSCHAFT FUR BIOTECHNOLOGISCHE FORSCHUNG.) 19 August 1982 see claims	40
Y	EP,A,0 433 258 (ENSO-GUTZEIT OY) 19 June 1991 see claims	40
Y	EP,A,0 429 422 (ENSO GUTZEIT OY) 29 May 1991 see claims	41
Y	EP,A,0 408 803 (ENSO-GUTZEIT OY) 23 January 1991 see claims	41
Y	EP,A,0 060 467 (EISENSTEIN) 22 September 1982 see claims	41
X	EP,A,0 504 005 (PERMA) 16 September 1992 see claims	42

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9201046	23-01-92	NONE	
WO-A-9216633	01-10-92	AU-A- 1430992 EP-A- 0575462 JP-T- 6505873	21-10-92 29-12-93 07-07-94
DE-A-3037992	19-08-82	US-A- 4432921	21-02-84
EP-A-0433258	19-06-91	JP-A- 3260188 NO-B- 174167	20-11-91 13-12-93
EP-A-0429422	29-05-91	CA-A- 2030186 JP-A- 3174078	18-05-91 29-07-91
EP-A-0408803	23-01-91	DE-D- 68912322 ES-T- 2061857 JP-A- 3130485 NO-B- 175105	24-02-94 16-12-94 04-06-91 24-05-94
EP-A-0060467	22-09-82	DE-A- 3110117 DE-A- 3128203	13-01-83 03-02-83
EP-A-0504005	16-09-92	FR-A- 2673534 JP-A- 6172145	11-09-92 21-06-94